Thesis for the Master's degree in Chemistry

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Synthesis of 6-arylpurines as potential selective antagonists for the various adenosine receptors

60 Study points

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After finishing a master degree in organic synthetic chemistry, everything in life seems so easy. With these words, my 6 years long "University of Oslo" chapter has come to an end.

ABSTRACT

Several 6-aryl-9-benzylpurines have been shown to be selective antagonists for various adenosine receptors including A_1, $A_{2A,}$ A_{2B} and A_3. The structures of the most active compounds resemble those screened in the *Mycobacterium tuberculosis (Mtb)* project in our group (Figure A). Some of these compounds were also screened for the adenosine receptors. The results from this screening and the exploration of structure-activity relationship (SAR) made the foundation for synthesizing new purine analogous as selective adenosine receptor antagonist. The target compounds were successfully synthesized with Stille and Suzuki coupling reactions and new effective methods have been developed. Herein the chemistry will be discussed.

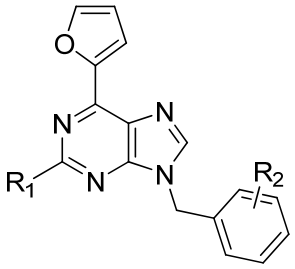


Figure A. Structure of target compounds. R_1 = Cl or NH_2. R_2 = F or Cl.

ABBREVIATIONS

AR	Adenosine receptor
Ac_2O	Acetic anhydride
BBB	Blood-brain barrier
Bu	Butyl
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
cAMP	Cyclic nucleotide adenosine 3',5'-monophosphate
DA	Dopamine
DMF	Dimethylformamide
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DMA	Dimethylacetamide
EWG	Electron withdrawing group
EDG	Electron donating group
EtOAc	Ethyl acetate
EtOH	Ethanol
GPCR	G-protein coupled receptors
GDP	Guanine diphosphate
GTP	Guanine triphosphate
HMQC	Heteronuclear Multiple Quantum Coherence experiment
HMBC	Heteronuclear Multiple Bond Correlation experiment
IUPAC	International union of pure and applied chemistry
KF	Potassium fluoride

Mtb	Mycobacterium tuberculosis
MS	Multiple sclerosis
Me	Methyl
MeOH	methanol
MS	mass spectroscopy
NMR	Nuclear magnetic resonance
n.d	not tested/ not determined
PD	Parkinson's Disease
PDE	Phosphodiesterase enzyme
r.t.	room temperature
SAR	Structure-activity relationship
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
UV	Ultraviolet light

CONTENTS

1. INTRODUCTION

"History suggests drug discovery is art as well as science".[1] During our time on this earth we have been using nature to cure illness. Ancient civilizations of Hindus, Chinese, Mayans of Central America and the Mediterranean people of antiquity hold the oldest records for the use of therapeutic plants and minerals.[2] Drug discoveries have a long tradition. Only the method and efficiency have changed. To design, synthesize and develop drugs is a long process. Considering the number of diseases without any specific drugs it is becoming more important to develop new chemical entities suitable for therapeutic use. Since the end of the nineteenth century countless drugs have been synthesized. The main reason for this was the progress in organic chemistry, which gave way for the pharmaceutical industry to bloom. Many peoples' lives have been saved and we have also extended our living time: *"The unprecedented increase in human life expectancy, which has almost doubled in a hundred years, is mainly due to drugs and to those who discovered them."* [2]

New drug discoveries require a considerable element of luck alongside with hard work. In this game where stakes are high, we are constantly seeking ways of improving our odds of being lucky in drug design. The first step is to look at the structure-activity relationship (SAR) [2] of the paharmacophore (recognition and binding geometries) and find potential lead compounds, which is a chemical structure that serve as a starting point for further chemical modification.[2] Good lead compounds have high potency[2], high selectivity[2], best pharmacokinetics [3] and are least toxic towards the normal cells in our body. In the course of evolution, several "designs" have emerged as preferred templates for certain functions. Just as the development of wings proved to be suited for flying, proteins and their ligands have evolved to perform specific biological functions.[1] By understanding these underlying principles, the drug-receptor interaction theory was evolved by John Newport Langley and later improved by Paul Ehrlich.[4]

1.1. THE BIOLOGIAL CONNECTION
~DRUG AND RECEPTOR

1.1.1 Receptor theory

A receptor is a molecular structure or site on the surface or interior of a cell that binds with substance such as hormones, neurotransmitters or drugs.[3] Receptors have many different functions in our body such as muscle contraction, regulation of growth factor, alternation of cellular morphology or function and gene activation.[2,5] Processes that lead to successful binding are generally called molecular recognition.[6] There are four major families of receptors that drugs are able to interact with. One of them is G-protein coupled receptors (GPCR). A_1, A_{2A}, A_{2B} and A_3 are adenosine receptor (AR) subtypes of the GPCR family.[2] They can be stimulated by potential drugs that work as an agonist or an antagonist (Figure 1). The potential of adenosine receptors as drug target was first reviewed in 1982.[7]

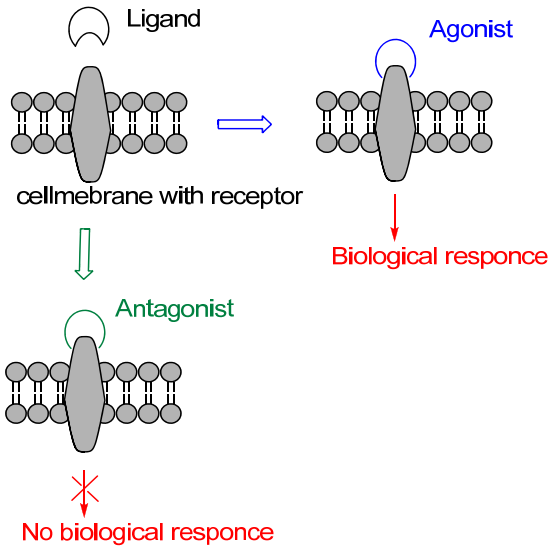


Figure 1. *Agonist*- binding lead to a certain biological response in the cell. *Antagonist*- inactivation of the receptor. No biological response.

1.1.2 Adenosine and the structure of G-protein coupled receptor

Adenosine (**1**) (Figure 2) is a ubiquitous purine ribonucleoside that exists in all cells, where it has important regulatory functions. It is a signalling substance, which mediates its effect by the activation of four receptor subtypes (A_1, A_{2A}, A_{2B} and A_3). All of them are members of the Class A, (rhodospin-like) family of the G-protein-coupled receptors (GPCR).[8] The estimated number of GPCR in human genome is ~30000 and this corresponds to more than 3% of the total number of genes.[9] They are large membrane bound proteins, which transduce their signal *via* the activation of an intracellular guanine nucleotide-binding protein (G protein). This family of proteins has seven hydrophobic domains that span the plasma membrane and possess alpha helical secondary structure (Figure 2).[2]

The extracellular region of the protein is composed of the N-terminus and three extracellular loops, which contain the ligand-binding site. The intracellular regions of the protein comprise the C-terminus and similarly three intracellular loops. The cytoplasmic side also includes the binding site of the G protein.[2,7] It should be noted that generally the transmembrane helices of the GPCR are uniform in size (20-27 residue), but their N- and C-terminal segments and the loops connecting their transmembrane helices vary widely in length with the identity of the GPCR (7-595 residue for the N- and C-terminus and 5-230 residue for the loops).[9] Structure of A_1 receptor and the possible interaction with adenosine is illustrated in Figure 2.[10]

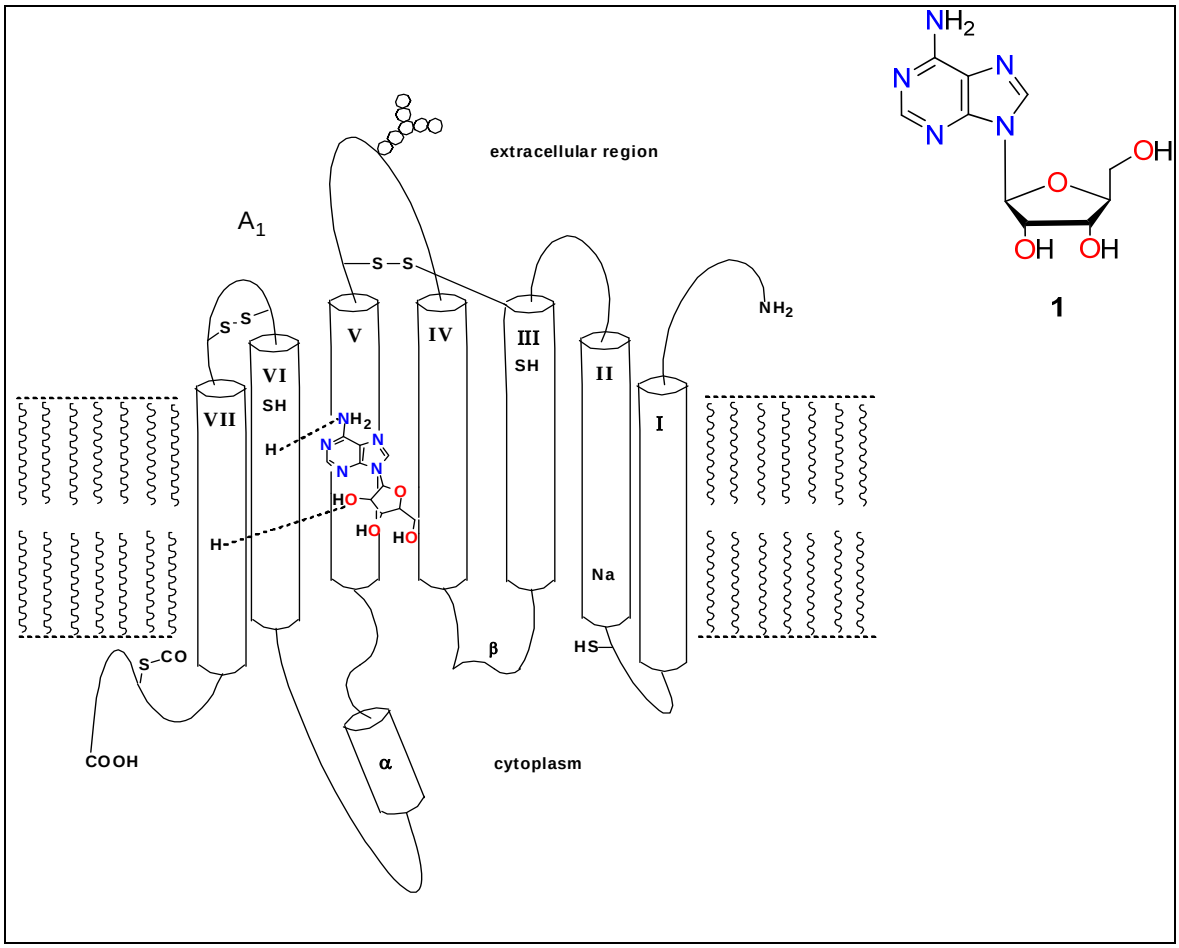


Figure 2.[9,10] Model of the A_1 adenosine receptor and adenosine **(1).** The seven transmembrane helices (I through VII) are arranged in a counter clockwise orientation.

1.1.3 The mechanism of G-protein coupled receptor

Generally the ARs are classified as inhibitors or activators of adenylyl cyclase activity. Usually the stimulation begins through binding of a ligand to a G protein-coupled receptor.[9] A schematic presentation of adenylyl cyclase activation *via* GPCR is shown in Figure 3.[11] The binding triggers adenylyl cyclase to synthesize cyclic nucleotide adenosine 3',5'-monophosphate (cAMP), which plays critical roles in mediating cellular signals in response to hormones, neurotransmitters, chemokines, and cytokines. The newly synthesized cAMP nucleotide binds and activates effectors, such as protein kinase

A. The protein kinase phosphorylates variety of substrates, like ion channels and transcription factors. The resulting signal can lead to changes in gene expression and cell metabolism. This will further lead to regulation of a wide variety of cellular functions. Some of them are immune responses, cardiac and smooth muscle contraction, visual response, apoptosis and growth control.[2]

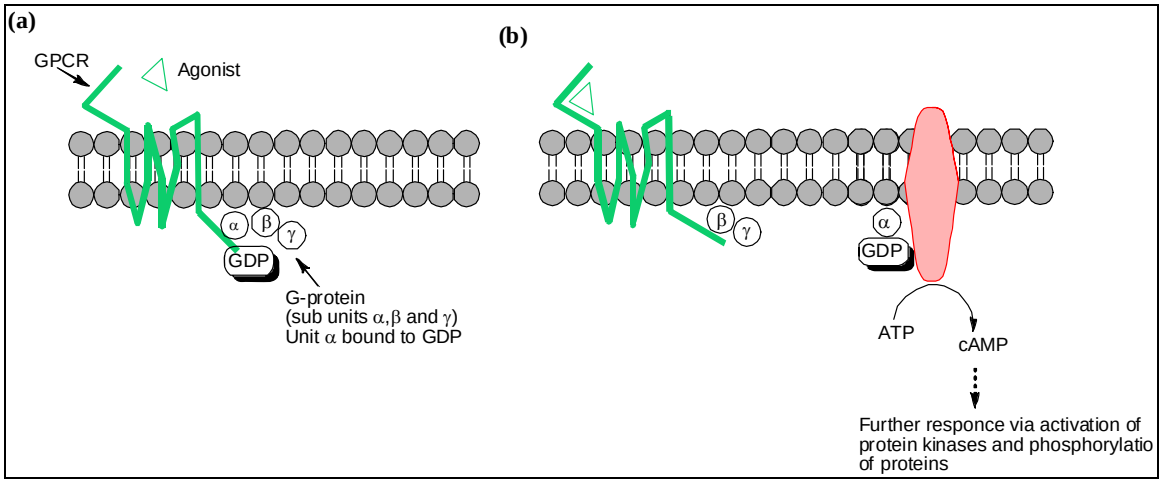
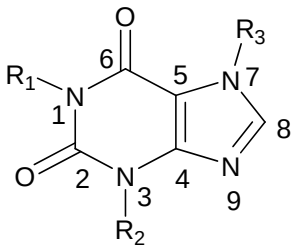


Figure 3.[11] Stimulation of adenylyl cyclase via G-protein coupled receptor (GPCR, green); **(a)** agonist approaching GPCR, guanine diphosphate (GDP) is bound to α-subunit of G-protein; **(b)** Binding of agonist to GPCR results in an exchange of GDP with guanine triphosphate (GTP). GTP + α-subunit moves to adenylyl cyclase (red) and stimulates the enzyme.

1.1.4 Xanthine antagonist for the adenosine receptors

Adenosine is unstable and has a small half life ($t_{1/2}$ <10s). Therefore, its application as a drug is limited, but it is used in certain heart diseases.[8] Xanthines were the first class of adenosine antagonist to be investigated and also the greatest explored.[12] Alkylxanthines are adenosine antagonists, which stimulate the activity of the central nervous system (CNS) and have been proven to be effective as cognition enhancers. This action leads to increased cerebral blood flow, that in turn increases glucose and oxygen availability to

the brain.[7] A naturally occurring class is metylxanthines, found in coffee (*coffea Arabica)*, cacao (*Theobroma cacao)* and tea (*camellia sinensis)*. The most important of them are caffeine and theophylline and they differ only by the position and number of methyl group on their xanthine ring system (Figure 4).[2]



	R_1	**R_2**	**R_3**
Caffeine	CH_3	CH_3	CH_3
Theophylline	CH_3	CH_3	H

Figure 4.[2] Structure difference between the methylxanthines. The numbers indicate the numbering system for purine.

Caffeine and theophylline is classical nonselective xanthine antagonist and display micromolar affinity for the AR subtypes. Caffeine is added to cola drinks as well as to pain stimulators. Theophylline is used as a bronchodilator in the treatment of asthma and chronic obstructive pulmonary disease (COPD). The bronchodilation is believed to occur through inhibition of the enzyme phosphodiesterase (PDE).[8] It has recently been shown that theophylline does inhibit PDEs in vitro, and x-ray crystallographic studies have identified the binding residues that interact with the methylxanthines (Figure 5). Theophylline binds to the active site (subpocket) *via* hydrophobic bonds. It seems like theophylline is sandwiched between a phenylalanine and a valine. Its binding affinity is strengthened by hydrogen-bonding between a tyrosine and N-7, and a glutamate and O-6 of the xanthine ring system.[2,13]

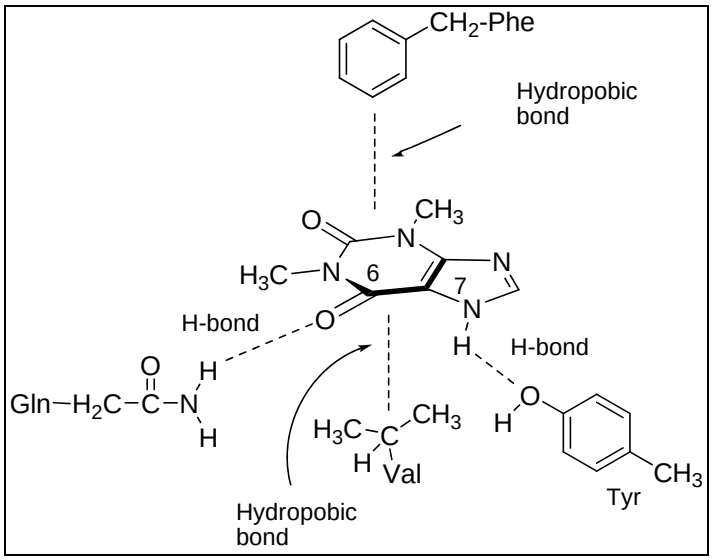


Figure 5.[2] Theophylline interactions in the catalytic pocket of PDE.

1.2 THE VARIOUS ADENOSINE RECEPTORS AND THEIR FUNCTION ~DETAILED DESCRIPTION

The stimulation by the binding of an agonist will lead to various intracellular responses. It is well known that more than one adenosine receptor can be expressed in a singe cell and this could lead to an atypical pharmacological response.[14] The distribution of the adenosine receptors in the body are show in Table 1.[7] There are many different diseases connected to each adenosine receptor subtype. Inflammatory conditions, sepsis, heart attack, ischemia-reperfusion injury, vascular injury, spinal cord injury, chronic obstructive pulmonary disease (COPD), asthma, diabetes, obesity, inflammatory bowel disease, retinopathy, and Parkinson's Disease (PD) are just a few examples.[8] New developments in medicinal chemistry for these receptors have enabled researchers to identify potential therapeutic areas for drug development.

Table 1.[7] Tissue distribution of adenosine receptor subtypes.

Adenosine receptor	**A_1**	**A_{2A}**	**A_{2B}**	**A_3**
Tissue Distribution	-Brain (cortex, hippocampus, cerebellum, thalamus) -Spinal cord -Testis -White adipose tissue, -Heart -Kidney	-Brain (striatum, nucleus accumbens, tuberculum olfactorium) -Heart -Lungs thymus -Spleen, -White adipose tissue	-Pars tuberalis (pituitary gland) -Large intestine -Bladder - Blood vessels	Species dependent; Human: -Lung -Liver -Placenta -Brain -Aorta -Kidney -Heart

1.2.1 Adenosine A_1 receptor ligands

The A_1 receptor is the best known and most studied AR subtype.[15] It is most concentrated in the brain,[16,17] but is also present in other tissues as well (see table 1). It is therefore essential that compounds targeted for A_1 is able to cross the blood-brain barrier (BBB).[12] Some of the diseases connected to A_1 receptor are asthma, inflammation, heart failure, hypertension and renal failure (kidney). Important classes of A_1-selective antagonist comprise xanthine derivates with bulky C-8 substituent and/or carrying a bulky substituent at N-6. Low water solubility of many A_1 antagonists remains a problem. Recent developments include the introduction of polar functions to increase the water solubility for an example, compound SW-13 (**2**).[15] Compounds N-0860 **(3)** and KW-3902 **(4)** are undergoing phase III development as agents for heart failure (Figure 6).[8]

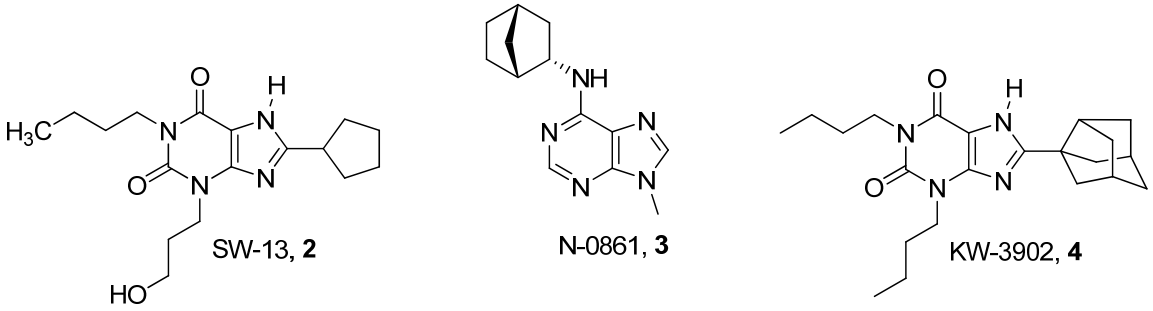


Figure 6. Selective A_1 antagonists.

1.2.2 Adenosine A_{2A} receptor ligands

Ischemia (blood supply), wound healing, sleep inducers, glaucoma (nerve) and Parkinson disease (PD) are some of the diseases connected to A_{2A} receptor. Dopamine (DA) modulates motor coordination and fine movements in our body.[18] Activation of the A_{2A} receptor in parts of the brain decreases the affinity of dopamine D_2 receptors for DA. Therefore there has been done extensive research in the last 10 years to develop potential drugs for treatment of PD and other neurodegenerative disorders.[8,14] Most of these compounds are chemically 8-styrylxanthines, like KW-6002 (**5),** currently in phase III for

PD treatment, has specific antagonist effect.[18,19] Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines like SCH-420814 (**6)**, in phase II (Figure 7).[8] It has also been found that 6-(2-furanyl)-9*H*-purine-2-amine derivatives have high A_{2A} receptor antagonist affinity. Benzyl substituent at *N*-9 with two fluorine atoms or a combination of fluorine and trifluoromethyl produce the best potency and selectivity (**7-9)** (Figure 7).[20] Similarly 6-aryl-9*H*-purin-9-ylcarboxamides derivates demonstrate high potency and selectivity towards A_{2B}. Compound **10** is an example.[21]

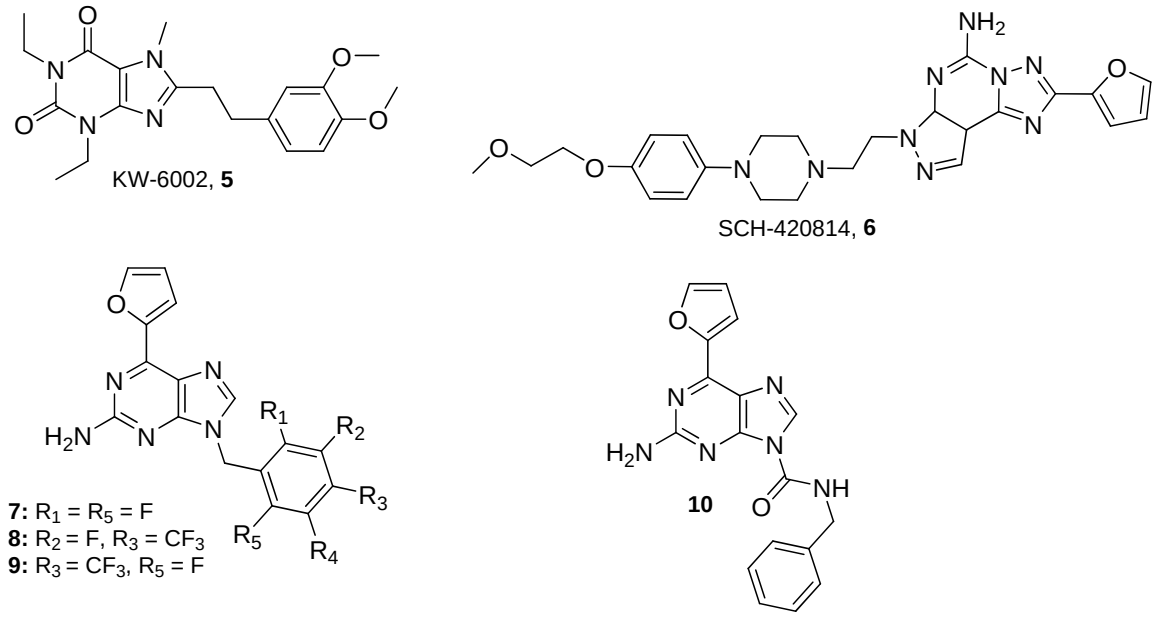


Figure 7. Selective A_{2A} antagonists.

1.2.3 Adenosine A_{2B} receptor ligands

The adenosine A_{2B} receptor is the least well characterized of the four adenosine subtypes, due to the lack of selective agonist and antagonist.[7,22] It was distinguished from A_{2A} due to its much lower affinity for adenosine. Adenosine is increased under abnormal conditions like under chronic pulmonary diseases, asthma, bowel diseases (intestine) [23] and other conditions. Selective A_{2B} are mostly xanthine derivates with substitution on the 8-position. A series of 8-pyrazole xanthine compounds have been found as selective A_{2B}

antagonist. One of them is CVT-6883 **(11)** and it is undergoing phase I development for treatment of asthma (Figure 8).[8] Further research has shown that 3-benzamide purine derivates like compound **12** has excellent A_{2B} affinity and good selectivity.[24]

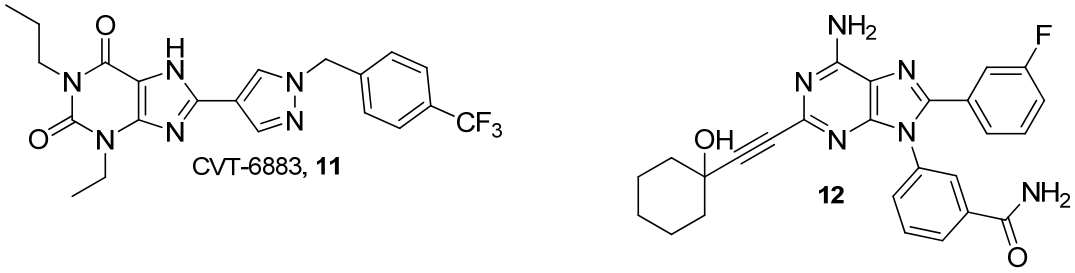


Figure 8. Selective A_{2B} antagonists.

1.2.4 Adenosine A_3 receptor ligands

A_3 adenosine receptors are the most recently discovered among adenosine receptors.[14] The A_3 AR is involved in many disease pathways like cancer, cardiac hypoxia, allergic conditions and Multiple sclerosis (MS). Natural antagonists such as caffeine and theophylline, show in general low affinity for A_3. Human A_3 selectivity is also found for several other classes of heterocycles like, xanthines, pyridines, flavonoids, thiazoles, thiadiazoles, isoquinolines and quinazolines.[8,25] There is a huge species difference in A_3 receptor binding affinity for xanthine antagonist.[7] 1-Benzyl-3-propyl-1*H*,8*H*-imidazole[2,1-*f*]purine-2,4-diones **(13)** is a highly potent and selective A_3 antagonist. Cyclization was made between the 7- and 8-position of the xanthine core (Figure 9). No selective A_3 ligand is currently undergoing clinical trials, but A_3 antagonist have been patented as inhibitors of tumor growth: MRE-3008-F20 (**14)** (Figure 9).[8]

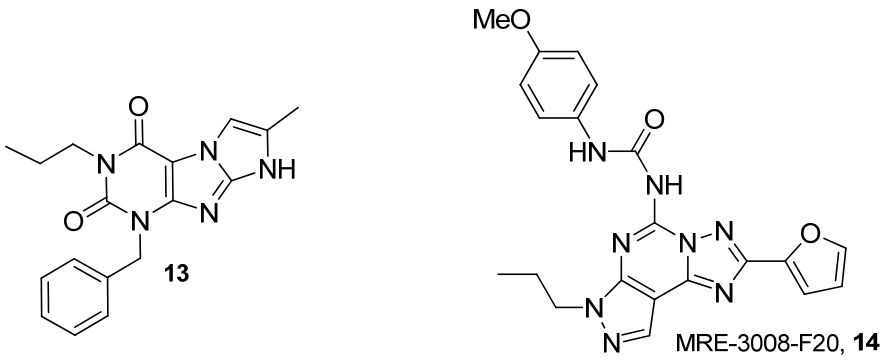


Figure 9. Selective A_3 antagonists.

1.3 DRUG DESIGN

1.3.1 Target molecules

The A_{2A} antagonist SCH 58261 **15** (Figure 10) has been reported to display nanomolar potency and modest selectivity (A_{2A} K_i = 4.3 nM, A_1/A_{2A} = 35).[20] Biological data in this thesis is presented as K_i values which is the dissociation constant for inhibitor binding.[26] The smaller value for K_i the better is the compound as inhibitor for adenosine receptors. Because of its low selectivity and poor solubility it has been used as a lead compound **(15)** in structure-activity relationship (SAR) investigation to find better target molecules. In compound **16**, the tricyclic core in SCH 58261 is replaced by purine ring, still carrying an amino-, a 2-furyl and an arylalkyl group. Several compounds **16** with different arylalkyl groups have been identified with high A_{2A} receptor antagonist affinity.[20]

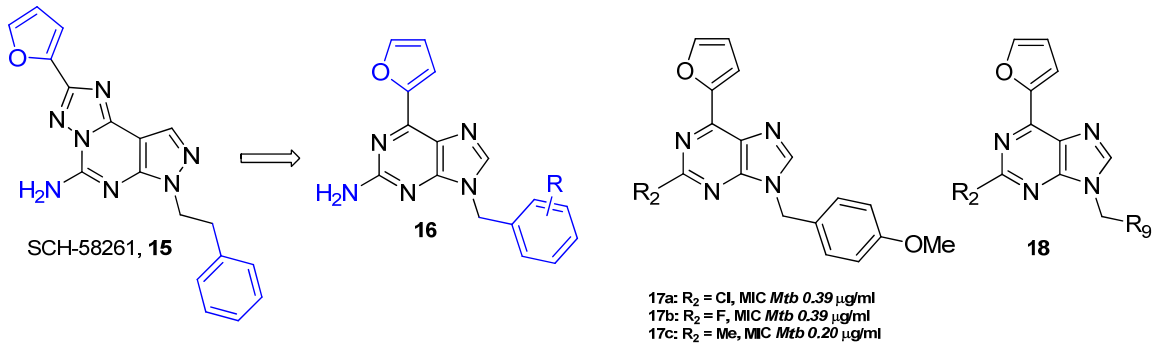
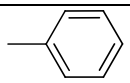
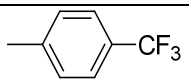
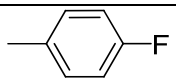
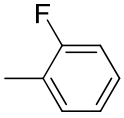
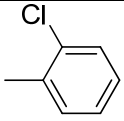


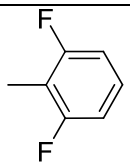
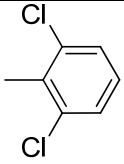
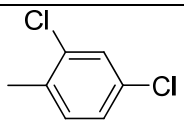
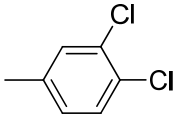
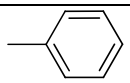
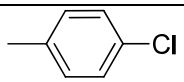
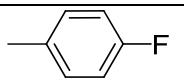
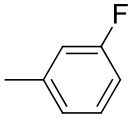
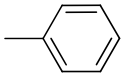
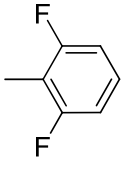
Figure 10.[11] Structural relationships between A_{2A} antagonists and antimycobacterial compounds.

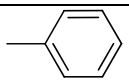
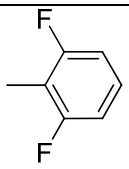
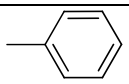
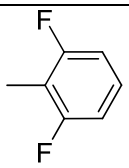
Compounds **16** are chemically closely related to 6-aryl-9-benzylpurines examined in our group as selective inhibitors for *Mycobacterium tuberculosis (Mtb) in vitro.*[27-36] Some of the most active antimycobacterial **(17)** are shown in Figure 10. The 6-aryl-9-benzylpurines developed in the antimycobacterial project in general display very low toxicity towards mammalian cells.[27-33] Therefore, a series of compounds **18** initially

prepared in the project directed towards antimycobacterial, have been screened as potential ligands for the four adenosine receptor subtypes. The screening has been carried out in the group of Professor Christa Müller at the University of Bonn. Approximately 60 target molecules of compound **18** have been screened and the result is the basis for the structures chosen as synthetic targets in this thesis. Table 2 gives an overview of some of the compounds that are significant in this thesis.[11]

Table 2.[11] Target molecules of compound **18** (Figure 10)**.**

Nr.	R_2	R_9	**K_i (μM)** or % inhibition at 10 μM value.					
			A_1 rat [^{3}H]CCPA	A_1 human [^{3}H]CCPA	A_{2A} rat [^{3}H]MSX-2	A_{2A} human [^{3}H]MSX-2	A_{2B} human (% inhib. at 1 μM)	A_3 human [^{3}H]PSB-11
18a	-H		> 10 (25 ± 1)	n.d	2.95 ± 0.81	n.d	> 1 (7 ± 2)	> 10 (28 ± 3)
18b	-H		> 10 (12 ± 1)	n.d	> 10 (37 ± 1)	n.d	> 1 (24 ± 1)	> 10 (32 ± 4)
18c	-H		> 10 (22 ± 3)	n.d	6.67 ± 1.17	n.d	> 1 (16 ± 4)	> 10 (27 ± 2)
18d	-H		> 10 (27 ± 6)	n.d	1.28 ± 0.14	n.d	≥ 1 (47 ± 1)	12.7 ± 0.7
18e	-H		> 10 (35 ± 2)	n.d	2.59 ± 0.93	n.d	>> 1 (0)	Ca. 10 (49 ± 1)

18f	-H		> 10 (42 ± 4)	> 10 (25 ± 6)	0.342 ± 0.088	0.302 ± 0.051	Ca. 1 (47 ± 2)	6.69 ± 1.18
18g	-H		> 10 (10 ± 1)	≥ 10 (48 ± 3)	0.443 ± 0.088	0.537 ± 0.036	> 1 (23 ± 3)	2.11 ± 0.78
18h	-H		> 10 (16 ± 0)	n.d	Ca. 10 (49 ± 0)	n.d	> 1 (10 ± 2)	2.98 ± 0.58
18i	-H		≥ 10 (40 ± 5)	n.d	6.11 ± 1.69	n.d	> 1 (21 ± 0)	≥ 10 (48 ± 1)
18j	-Cl		> 10 (39 ± 4)	> 10 (45 ± 6)	> 10 (34 ± 4)	1.74 ± 0.22	> 1 (1 ± 3)	0.80 ± 0.215
18k	-Cl		> 10 (10 ± 2)	n.d	> 10 (25 ± 6)	n.d	> 1 (24 ± 2)	3.69 ± 1.25
18l	-Cl		> 10 (39 ± 2)	n.d	2.199 ± 0.532	n.d		
18m	-Cl		> 10 (12 ± 2)	n.d	> 10 (22 ± 2)	n.d	> 1 (17 ± 3)	1.12 ± 0.16
18n	-NH_2		0.997 ± 0.253	n.d	0.111 ± 0.020	n.d	0.315 ± 0.039	2.90 ± 0.25
18o	-NH_2		1.360 ± 0.190	n.d	0.138 ± 0.037	n.d	n.d	n.d

18p new	-NHCOMe		1.11 ± 0.19	5.92 ± 0.37	0.264 ± 0.067	0.089 ± 0.011	0.0111 ± 0.012	0.0703 ± 0.0249
18q new	-NHCOMe		1.510 ± 0.120	n.d	0.321 ± 0.069	n.d	n.d	n.d
18r new	-$N(COMe)_2$		1.73 ± 0.09	> 10 (26 ± 8)	1.24 ± 0.295	0.677 ± 0.11	≥ 1 (44 ± 4)	0.205 ± 0.008
18s new	-$N(COMe)_2$		0.417 ± 0.030	n.d	0.247 ± 0.030	n.d	n.d	n.d

The potency and the selectivity for the adenosine receptors is dependent on whether there is an electron withdrawing group (EWG), electron donating group (EDG) or a combination of both on R_2 **(18a-18s)**. A phenyl ring is required at R_9 **(18a-18s)** and potency and selectivity is influenced by the position (ortho, para and meta) and the number of halogens on the aromatic ring.

From table 2 it is possible to describe some of the important chemical features that are essential for good selectivity and potency for the various ARs:

- **A_1 AR:** A_1 has poor potency until an EDG group or a combination of EWG/EDG is introduced at R_2. Diacetamide on R_2 and fluoride(s) on ortho position on phenyl are required. Compound **18s** has the best potency, but a very poor selectivity compared to A_{2A}. Fluorides on the phenyl ring are appreciated in the A_1 receptor binding pocket, because when they are removed the total affinity drops by 1.31 μM **(18r & 18s).**

- **A_{2A} AR:** Several compounds show good potency for A_{2A} with EWD on R_2, but better potency is received when amino, acetamide or diacetamide is introduced (EDG or EWD & EDG). The difference between acetamide and diacetamide is huge for A_{2A} human receptor **(18p & 18r).** Compound **18n** display the best potency and selectivity compared to A_1, A_{2B} and A_3. For A_{2A} human receptor, chlorides are evidently less appreciated than fluorides **(18f & 18g).** This indicates a strong electron donating amino acid residue in the receptor sup pocket. The fluoride will function as a hydrogen-bond acceptor.

- **A_{2B} AR:** A EWG group like amino or combination of EWG and EDG like acetamide is required on R_2. This is similar to the A_1 AR. Acetamide at R_2 gives a much higher affinity for A_{2B} receptor, compared to amino **(18n & 18p).** Compound **18p** show the best potency and selectivity compared to A_1, A_{2A} and A_3.

- **A_3 AR:** Compound **18j** with a EWG (-Cl) at R_2 has the best selectivity and good potency compared to the other AR antagonists. But **18p** has the best potency, but poor selectivity compared to A_{2B} AR. Affinity for the other receptors increases when R_2 has EDG or a combination of EWG and EWG. Despite this, acetamide on R_2 has 64% more affinity for A_{2B} AR, compared to the diacetylated antagonist **(18p & 18r).** Chlorides seem to be more accepted on the aromatic ring compared to fluorides **(18f & 18g).** This is opposed for A_{2A} human receptor.

Even though some of the data, were the halogen are attached to phenyl, is missing for A_{2A} -, A_{2B} -, A_3 AR, it seems like the halogens have a key role in the binding geometry for the AR antagonist (table 2). AR compounds **(18a-18s)** generally gain better affinity when fluoride(s) is present, compared to chloride(s). It is well known that fluorides are used to further improve the physico-chemical properties of potential drugs, often with dramatic impact on biological properties. It has been estimated that up to 20% of new drugs released contain fluoride.[37]

1.4 THE CHEMISTRY

All starting materials were prepared by *N*-alkylation of purines. Stille and Suzuki coupling are the methods applied for introducing C-C bond on the *N*- alkylated purines 6-position. Some of these compounds have further been subjected to acetylation.

1.4.1 Purine chemistry

Purine **(19)** is the most abundant nitrogen-containing heterocycle on earth (Figure 11).[38] It is a colourless, crystalline compound which is characterized as a weak base.[39] Most of the naturally occurring purines have at least oxygen or nitrogen incorporated in the ring system. Purine itself is not found in nature, and was first prepared by Emil Fischer,[39] but the bicyclic ring system is found in many naturally occurring compounds with a wide range of biological roles.[38]

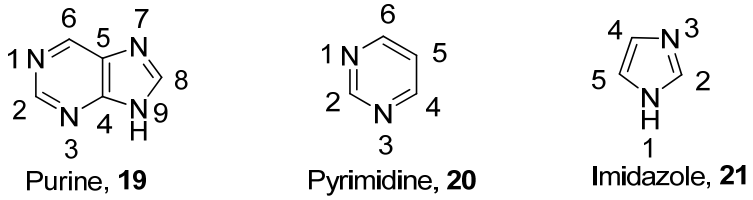
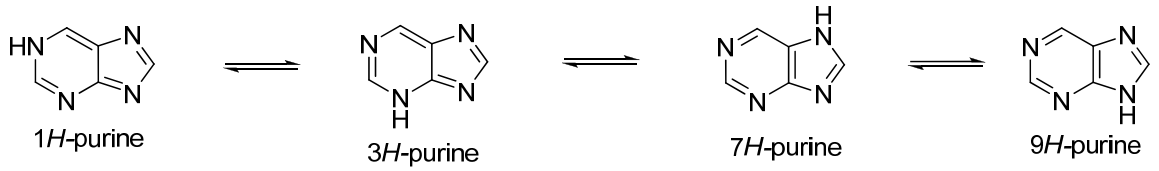


Figure 11. The name purine goes back to the Latin words purum (pure) and uricum (urine).[39] Herein the purines will be numbered according to this model, following the IUPAC convention.

In principle purines can exist in four different tautomers containing *NH*-hydrogen (Scheme 1). According to quantum mechanical calculation, the 1*H*- and the 3*H*-tautomers are much less stabile than the other two isomers.[39] The most significant are 7*H*- and 9*H*-

tautomers. They are present in solutions and the tautomer distribution is dependent on the nature of solution and temperature.[40]

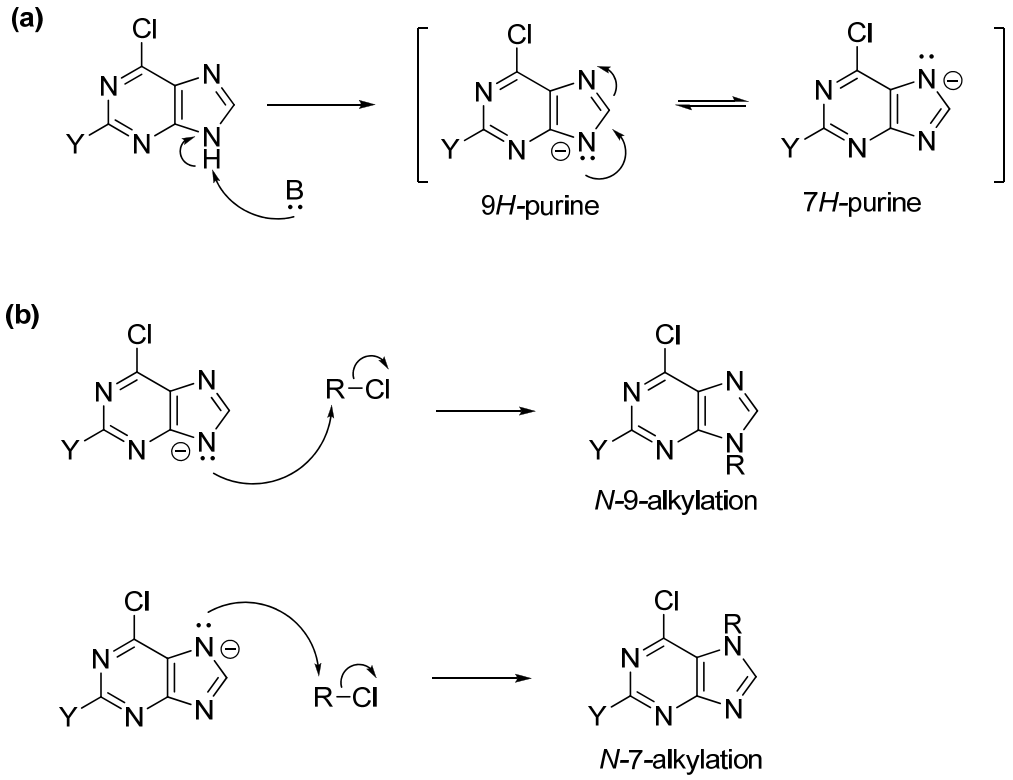


Scheme 1. Purine tautomers.

Purines bicyclic ring system has 10 π-electrons and composes a 5- and 6-ring. The 6-ring is derived from pyrimidine **(20)** and the 5-ring from imidazole **(21)** (Figure 11).[40] Pyrimidine can be compared to benzene in certain chemical properties. Both molecules have 6 π-electrons. Benzene has an even distribution of electrons, whereas pyrimidine has electron deficient carbons due to higher electron negativity of the nitrogens, and the carbons will easily undergo nucleophilic substitution. [41] If the pyrimidine part on the purine has a good leaving group (e.g. a halogen) the molecule will be considerably more reactive. The imidazole ring has 6 π-electrons distributed in a 5-ring. Because of this high electron density, electrophilic aromatic substitutions will rather occur on the imidazole ring. As a result of purines fused chemistry, nucleophilic substitutions can take place on both rings, while electrophilic substitutions can only be done on the 5-ring.[40]

1.4.2 *N*-alkylation

In *N*-alkylation a chemical entity is substituted on the purine heteroatom (Scheme 2). The substituent used herein is benzyl derivate with flour or chloride.



Scheme 2. *N*-alkylation of 6-chloropurine, Y = Cl or NH_2; **(a)** B = K_2CO_3. The base removes hydrogen and resonance structures of two anions are formed; **(b)** R = benzyl derivate with flour or chloride. The alkyl halide (R-Cl) react and to isomers are formed.

A lot of starting materials were synthesized early in our group utilised *N*-alkylation reaction with purine[27-33,42-44] and they normally give two isomers. It is not possible to achieve complete selectivity. Substituent on purine has an effect on the isomer distribution in a way that the *N*-9-alkylation is formed in highest yield and is the main

product. Chloride is good leaving group and the isomers are separated by flash chromatography.[45] The *N*-9-alkylated product is further subjected to Stille or Suzuki coupling.

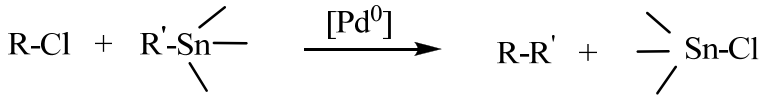
1.4.3 Coupling reactions: in general

Cross-coupling reactions are widely used and efficient way of constructing C-C bonds. Treatment of purines containing suitable leaving groups (halogen) with diverse type of organometallic compounds like Mg, Cu, Al, Zn, Sn and B has been extensively studied and several efficient preparative methods for the synthesis of various types of 2-, 6-, and 8-C-C-purines have been developed.[27,46] The order of reactivity of aryl halide in palladium mediated cross coupling reactions is found to be Ar-I > Ar-Br >> Ar-Cl. 6-chloropurines (Scheme 2) have earlier in our group shown to be reactive enough to give high yields of coupling products in palladium catalyzed reactions. In addition iodopurines are less commercially available.[33] For these reasons 6-chloropurines was chosen. The Suzuki reaction is, together with the Stille reaction, currently the most popular method for the introduction of aryl or heteroaryl substituent in the purine 2-, 6-, or 8-position. The choice of method is often governed by the availability of the desired organometallic coupling partner. The Suzuki coupling may be regarded as more environmentally friendly, compared to the Stille coupling were organotin compounds are employed (Scheme 5).[38]

1.4.4 Stille coupling

The stille reaction has established itself as one of the most general and most selective palladium-catalyzed cross-couplings reactions.[47] In 1978 John K. Stille and his co-workers carried out extensive synthetic and mechanistic work that made this reaction a standard method in organic synthesis. The Stille reaction rank today as the more general transformations in organic synthesis. Stille coupling is used in synthesizing complex molecules and it display high selectivity and broad scope. In addition Stille coupling has

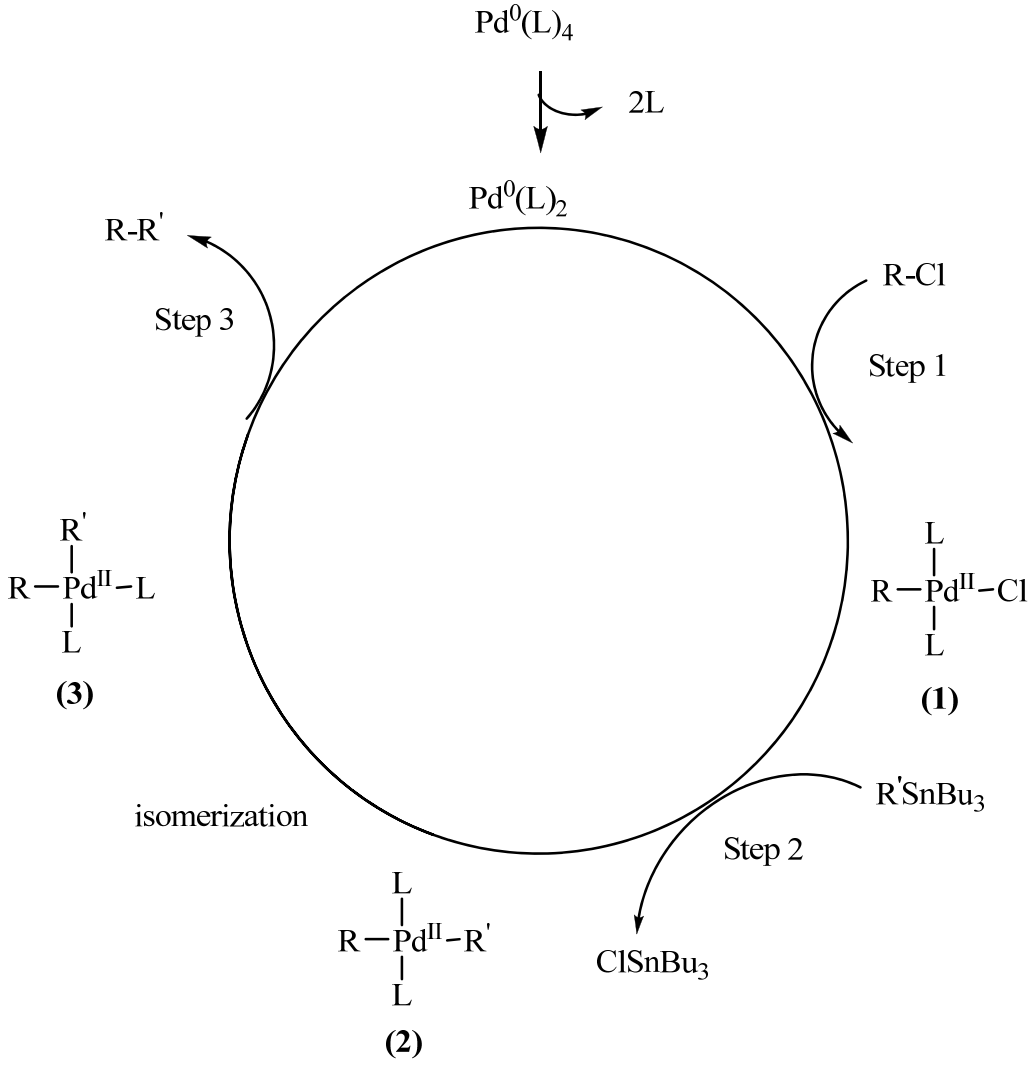
a good tolerance toward most functional groups. This makes it particularly effective for transformation of highly functionalized molecules. The main advantage is that sp, sp^2 and sp^3 hybridized carbons can be coupled for the formation of a C-C bond (Scheme 3).[48] This method has in fact been applied successfully to the construction of a variety of purine ring system bearing sensitive functional groups.[27-33,38,49-53]



Scheme 3. Stille Coupling involves reaction of organostannane with an organohalide. R = C.

1.4.4.1 Mechanism[47]

The active compound $Pd^0(L)_2$ react with the organic electrophil, R-Cl. Step 1 is called oxidative addition were complex **1** is formed (Scheme 4). Palladium undergoes oxidation from 0 to II. A transmetallation occur in step 2. An organometallic compound will exchange the halide with an alkyl group. During this process complex **2** is formed, but it will undergo rapid transformation from *trans* to *cis.* This is called an isomerization reaction and lead to the formation of complex **3**. In the third and last step, two alkylgroups (R-R') will be reductive eliminated and form a new C-C bond. Palladium will simultaneously change oxidation number from II to 0 and the complex is now regenerated.



Scheme 4[47]. Catalytic cycle. Cl is the outgoing group. L = catalyst ligand. R-R' is the new compound formed in the reaction.

The major downfall for the Stille coupling reaction is the high toxicity of the trialkyl organostannane ($ClSnBu_3$) formed in the reaction. Separation of the organotin from the desired product cannot be achieved because both are soluble in nonpolar organic solvent. In contrast to organotinchlorides and organotinbromides, the triaryltinfluorides are insoluble in organic solvents.[54] When a solution of potassium fluoride (KF) in an organic solvent is added to the reaction mixture, the $ClSnBu_3$ will be converted to the insoluble

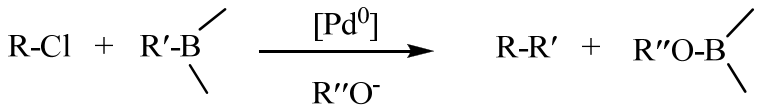
$FSnBu_3$ (Scheme 5). The tributyltin chloride can also be removed by dissolving the crude product in acetonitrile and extracting the tin chloride with hexane.[33] Previously in our group organostannanes have been used to synthesize purine analogues.[27-33,49-52] Successful results have been achieved and therefore these conditions were applied in the Stille coupling reactions.

$$ClSnBu_3 + KF_{(aq)} \rightarrow FSnBu_{3(s)} + KCl_{(aq)}$$

Scheme 5. Converting organotin with potassium fluoride.

1.4.5 Suzuki coupling

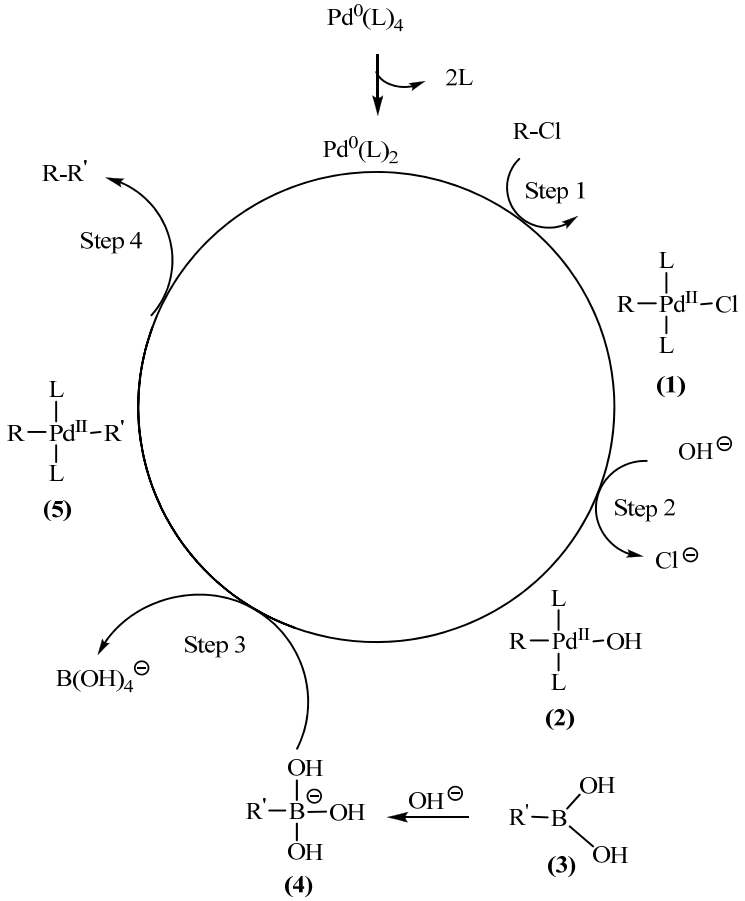
The palladium-catalyzed cross-coupling reaction between organoboron compound and organic halides is called Suzuki coupling (Scheme 6). This is a powerful and general methodology for the formation of carbon-carbon bonds.[38] The availability of the reagents and the mild reaction conditions contribute to the versatility of this reaction. Suzuki coupling has several advantages, such as being largely unaffected by the presence of water, tolerating a broad range of functional groups and proceeding generally regio- and stereoselectivity. Additionally, the inorganic by-product of the reaction is non-toxic and easily removed from the reaction mixture, making the Suzuki coupling suitable not only for laboratories but also for industrial processes.[48,55] Numerous purine derivates have been synthesized using this method.[31,56-58]



Scheme 6. Suzuki couplings involve reaction of organoboron with an organohalide.

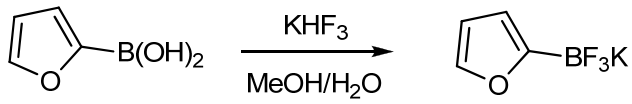
1.4.5.1 Mechanism[55,59]

The first step is similar to Stille coupling, were the oxidative addition of the halide (R-Cl) form the organo-palladium species **(1)** (Scheme 7)**.** Further reaction with a base gives the intermediate **2** and the halide is cleaved off (step 2). The organoboron **(3)** must be activated with base for the reaction to proceed. This is the difference between Suzuki mechanism and Stille coupling. The activated compound **4** is called "ate" complex. This negatively charged ate complex undergo a clean coupling reaction with compound **2** via transmetalation and the second organo-palladium species **(5)** in this reaction is formed (step 3). Reductive elimination gives the desired product (R-R') and restores the original palladium catalyst.



Scheme 7. Catalytic cycle. Cl is the outgoing group. L = catalyst ligand. R-R' is the new compound formed in the reaction.

The boron reagent used in the Suzuki coupling is potassium triflouroborate and they were previously synthesized in our group. They are easily prepared from organoboronic acids by treatment with an aqueous solution of KHF_2 (Scheme 8).[60]

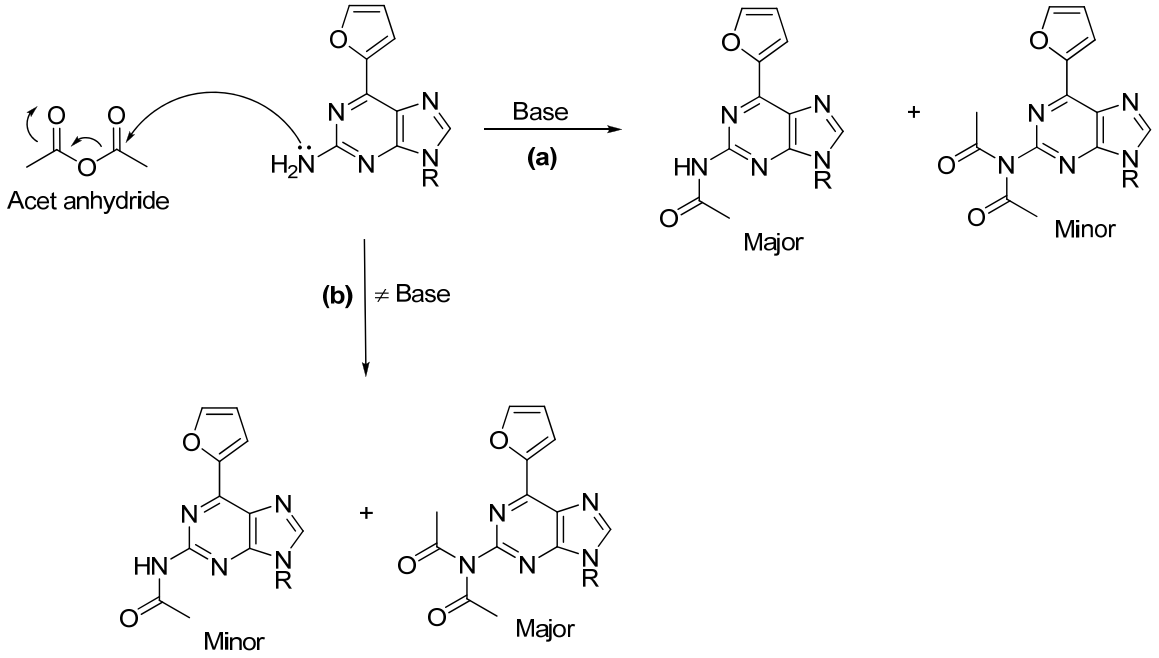


Scheme 8. Synthesis of potassium furan-2-yltrifluoroborat

Potassium furan-2-yltrifluoroborat is stable in air, environmentally friendly and works very well in Suzuki coupling. The byproduct from this reaction is converted to harmless inorganic salts that are readily separable from the desired product.[60] Suzuki coupling was performed on the compounds that had been subjected to Stille coupling. The purpose was to see which coupling works best in order of yield, reaction time and environmental and health issues.

1.4.6 Acetylation of amine

Some of the coupling products were subjected to acetylation of amine in the purine 2-position (Scheme 9). Acetic anhydride reacts with amine to form amide. The acetylation can be performed in organic solvent in the presence of a base. [41] Herein triethylamine has been used,[32,61] but other bases like pyridine is also common.[32,62] In these reactions the monoacetylated product is formed as a major product. Only acetic anhydride is used without base to shift the ratio to produce diacetylated compound in major amount. .[32,63]



Scheme 9. Acetylation of amine on purine 2-position, R = benzyl derivate with flour or chloride; **(a)** Monoacetylation; **(b)** Diacetylation.

2. SYNTHESIS OF MOLECULES

Compounds **27a, 28a, 29a, 30a, 30b, 31a** and **31b** were the target molecules. This section discusses in detail how they were synthesized.

- **N-alkylation**

- 2,6-Dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a)** and 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b)**
- 6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** and 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b)**
- 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** and 6-chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b)**

- **Stille vs. Suzuki coupling**

- 2-Chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine (**27a)**
- 9-(4-Chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine (**28a)**
- 9-(4-Fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)**

- **Acetylation of amine**

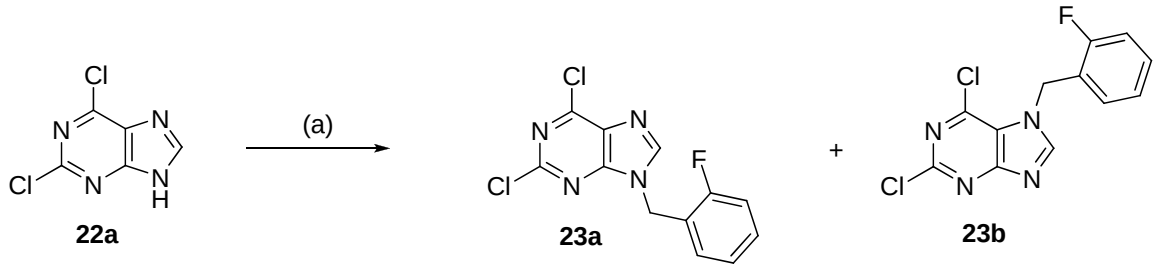
- *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a)** and *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)**
- *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)** and *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b)**

2.1 N-alkylation

Six different *N*-alkylated compounds were made. **23a**, **25a** and **26a** served as starting material. These were synthesized because *N*-9 alkylated compounds have been reported as adenosine receptor antagonist,[20] while *N*-7 alkylated compounds have not been reported as AR antagonist.

2.1.1 Synthesis of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine (23a) and 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine (23b)

2,6-Dichloro-9-(2-fluorobenzyl)-9*H*-purine (**23a)** was isolated in 61 % yield, together with 19 % of the *N*-7 benzylated isomer **(23b).** This was done by alkylation of 2,6-dichloropurine[27] **(22a)** with 2-fluorobenzyl chloride in DMF employing potassium carbonate as base at room temperature (Scheme 10).[50]



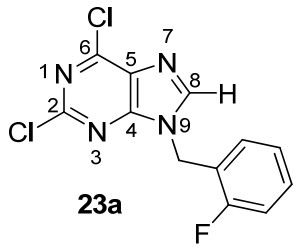
22a **23a** **23b**

Scheme 10. Reagents and conditions: (a) 2-fluorobenzyl chloride, K_2CO_3, DMF, 20 hours at r.t.

The ^{1}H NMR spectrum of the crude product showed the ratio distribution of isomers to be 5:1 **(23a:23b)**, were the *N*-9 isomer was the dominant of the two. This regioisomeric ratio has been reported earlier for *N*-9/*N*-7 alkylation.[42] When DMF is used as solvent the

N-9 isomer yield is normally higher, compared to when DMSO or DMA are used as solvents.[49] The staring material was completely converted to products, judged by crude ^{1}H NMR and TLC. Since compound **23a** was used in further reactions, this is indeed good condition. In order to purify both isomers from each another, two attempt of flash chromatography were required. Combination of ethyl acetate:hexane (1:1) was used as solvent. They were differentiated by HMQC and HMBC NMR experiment. Table 3 give some of the selected correlation from long range HMBC spectrum.

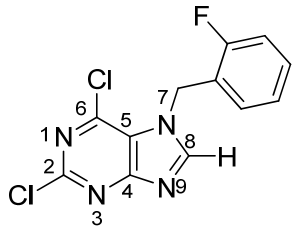
Table 3. Selected correlation from long range HMBC spectrum for the *N*-9 isomer (**23a).** X = coupling between carbon and hydrogen.



23a

	C-4	**C-5**	**C-8**
CH_2	X		X
H-8	X	X	

Table 4. Selected correlation from long range HMBC spectrum for the *N*-7 isomer (**23b).** X = coupling between carbon and hydrogen.



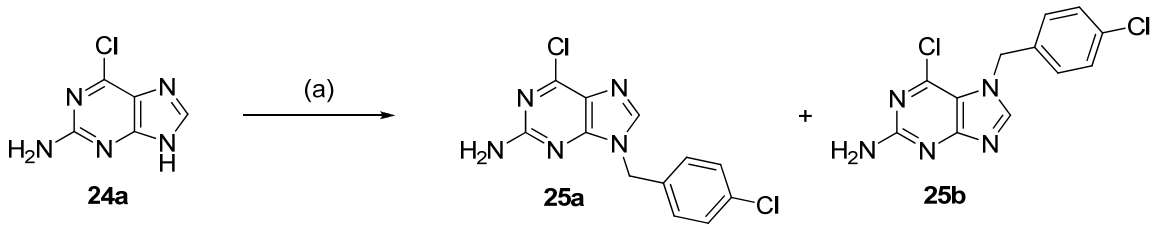
23b

	C-4	**C-5**	**C-8**
CH_2		X	X
H-8	X	X	

Couplings from carbons C-4 and C-5 clearly verify each isomer. C-4 on *N*-9 compound **(23a)** couples to CH_2 and H-8, whereas C-5 has the same correlation for the *N*-7 molecule **(23b).** Carbon 2 and 6 was differentiated by the number of heteroatom they were bound to. C-2 has two nitrogen and one chloride attached and this indicate a higher shift value compared to C-6.[27,49] The other carbons were identified by direct correlation of hydrogen to the attached carbon (HMQC).

2.1.2 Synthesis of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine (25a) and 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine (25b)

2-Amino-6-chloropurine[28,60] **(24a)** was *N*-alkylated with 4-chlorobenzyl chloride (Scheme 11). The reaction condition is the same as described above.



Scheme 11. Reagents and conditions: (a) 4-chlorobenzyl chloride, K_2CO_3, DMF, 23 hours at r.t.

Optimization of the reaction condition for synthesis of compounds **25a** and **25b** is given in Table 5, entries 1-6.

Table 5. Optimization of the reaction condition for synthesis of compounds **25a** and **25b**.

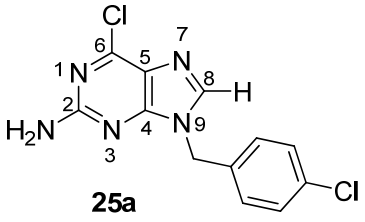
Entry	**Compound**	**Ratio crude ^{1}H NMR (25a:25b)**	**Amount (mg)**	**Yield (%)**	**Flash chromatography Solvent system**
1	25a	n.d	268	18	EtOAc/Hexane (2:1)
	25b		-	-	
2	25a	n.d	437	30	EtOAc/Hexane (2:1) then (1:0)
	25b		-	-	
3	25a	10:1	930	63	EtOAc/Hexane (2:1) then (1:0)
	25b		-	-	
4	25a	7:1	190	13	DCM/MeOH (95:5)
	25b		-	-	
5	25a	n.d	770	52	DCM/MeOH (98:2) then (94:6)
	25b		202	15	
6	25a	4:1	910	62	DCM/MeOH (98:2) & (90:10)
	25b		200	14	

For entries 1 and 2 (Table 4) the minor *N*-7 isomer was not present in the crude ^{1}H NMR or TLC. If *N*-7 alkylated products were formed, they were in quantity to low to be isolated. Because the compounds were slightly soluble in ethyl acetate/hexane, it resulted in poor yields. Compounds **25** have an amino group in the purine 2-position and they are highly polar compared to compounds **23**. *N*-7 is more polar compared to *N*-9 and there is

a huge distance for the spots on TLC. *N*-7 isomer is only isolated when a combination of DCM/MeOH is used as solvent (entry 5 and 6, Table 4). Still it is possible to isolate a considerable amount of **25a** with ethyl acetate/hexane, as entry 3 indicates. But this separation required a lot of solvent, because of the poor solubility of compounds **25a** and **25b.** Entry 4 gave the lowest yield, and even though polar solvents were used *N*-7 isomer was not isolated. A small column was tested to see whether a quicker separation could be achieved, but this gave insufficient results. The ratio of *N*-9/*N-7* in entry 5 is not determined, because of no data available.

The isomers of compound **25** were also distinguished by HMQC and long range HMBC NMR experiments. The correlations are exactly the same as for compounds **23.** This is illustrated in table 5 were C-4 and C-5 correlations prove the difference. Carbon 2 and 6 was differentiated by the number of heteroatom they were bound to. C-2 has two nitrogen and one amino group attached and will therefore have higher shift value than C-6.[32]

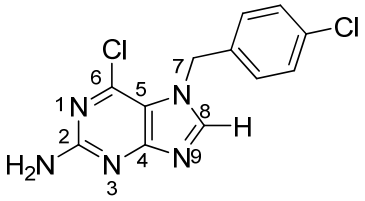
Table 6. Selected correlation from long range HMBC spectrum for the *N*-9 isomer (**25a).** X = coupling between carbon and hydrogen.



25a

	C-4	**C-5**	**C-8**
CH_2	X		X
H-8	X	X	

Table 7. Selected correlation from long range HMBC spectrum for the *N*-7 isomer (**25b).** X = coupling between carbon and hydrogen.

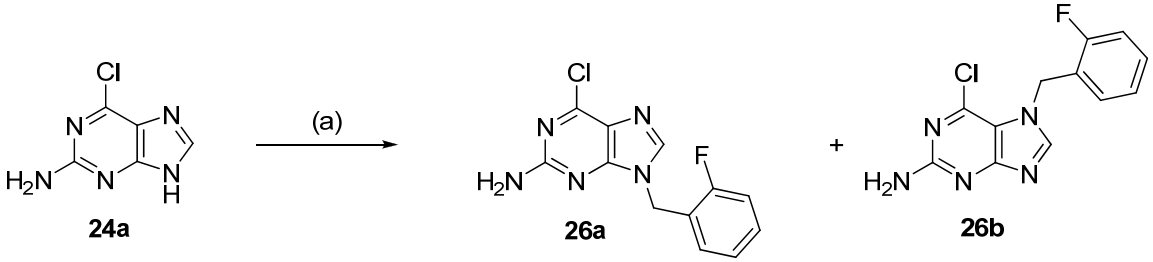


25b

	C-4	**C-5**	**C-8**
CH_2		X	X
H-8	X	X	

2.1.3 Synthesis of 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine (26a) and 6-chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine (26b)

2-Amino-6-chloropurin[28,64] **(24a)** was reacted with 2-fluorobenzyl chloride to give the two *N*-alkylated isomers 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine (**26a)** and 6-chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine (**26b)**, respectively (Scheme 12). For reaction mechanism, see Scheme 2.



24a **26a** **26b**

Scheme 12. Reagents and conditions: (a) 2-fluorobenzyl chloride, K_2CO_3, DMF, 18 hours at r.t.

The reaction condition was the same as for the synthesizing of compounds **23** and **25.** Yields and amounts are given in Table 8.

Table 8. Optimization of the reaction condition for synthesis of compounds **26a** and **26b**.

Entry	**Compound**	**Ratio crude ^{1}H NMR (25a:25b)**	**Amount (mg)**	**Yield (%)**	**Flash chromatography Solvent system**
1	26a	11:1	836	60	DCM/hexane/EtOAc (1:1:2) then (0:0:1)
	26b		-	-	
2	26a	5:1	706	51	DCM/MeOH (95:5) then (90:10)
	26b		64	5	
3	26a	n.d	780	56	DCM/MeOH (95:5) then (90:10)
	26b		30	2	
4	26a	10:1	940	68	DCM/MeOH (95:5) then (90:10)
	26b		80	6	
5	26a	n.d	960	69	DCM/MeOH (95:5) then (90:10)
	26b		100	7	

Here purine has an amino group in the 2-position as well. A mixture of DCM/hexane/EtOAc was not polar enough to isolate the *N*-7 isomer (entry 1, Table 6), even though it appeared on crude ^{1}H NMR. But the yield of product **26a** is good and the only disadvantage being the use of huge amount solvent during flash chromatography, again due to solubility issue. The time may also be considered as an issue, because the

efficiency is lowered. The best yields of compound **26a** were achieved when a combination of DCM/MeOH was applied as solvent (entries 4 and 5, Table 6). Improvement in separation technique resulted in higher yield compared to entries 2 and 3. Flash chromatography was carried out twice for entry 2 in order to achieve best separation of the two isomers.

Generally yields for the different isomers described here is within the range of what have been reported before.[27,43,49,50] This is also the applicable for compounds **23** and **25**. Even though compound **26a** cannot be synthesized with complete selectivity, reaction condition can be optimized to increase the ratio of *N*-9 isomer compared to *N*-7. It is also worth noticing that *N*-9 isomer is always exclusively higher than *N*-7 alkylated product. The ratio of *N*-9/*N*-7 in crude ^{1}H NMR for entries 3 and 5 is not determined, because of no data available.

Characterisations of the two isomers **(26)** were done in the same manner as for products **25.** For long range HMBC correlation see Table 5. It is also possible to see the difference between the isomers by looking at where the shift values for certain carbons and hydrogen appear on NMR spectra. Table 9 gives a comparison of ^{1}H and ^{13}C NMR shift values for compounds **23a, 23b, 25a, 25b, 26a** and **26b.**

Table 9. Comparison of ^{1}H and ^{13}C NMR shift values for compounds **23a, 23b, 25a, 25b, 26a** and **26b.**

Compound	**Isomer**	**^{1}H NMR**		**^{13}C NMR**		
		H-8	**NH_2**	**CH_2**	**C-5**	**C-8**
23a	*N*-9	8.79	-	41.4	130.4	148.3
23b	*N*-7	9.00	-	44.2	121.9	153.0
25a	*N*-9	8.21	6.93	45.4	123.3	143.1
25b	*N*-7	8.55	6.67	48.4	114.6	149.8
26a	*N*-9	8.14	6.92	40.1	123.4	143.2
26b	*N*-7	8.50	6.67	44.6	115.7	151.1

The trend is now clearly seen from table 7. H-8 signals for *N*-9 isomers are shifted upfield to the corresponding H-8 signals for *N*-7 isomers. But the NH_2 signals for *N*-9 isomers (**25** and **26**) are shifted downfield to the corresponding NH_2 signals for *N*-7 isomers. The ^{13}C NMR signals for CH_2 and C-8 for the *N*-9 isomers are shifted upfield and shielded compared to the *N*-7 alkylated compounds. The most significant difference in ppm values is seen in C-5. They are deshielded relative to *N*-7 isomers. Similar ^{13}C shift differences have been reported for hypoxanthine, adenine, 6-mercaptopurine regioisomeric derivatives and other purine compounds.[39,65]

2.2 Stille vs. Suzuki coupling

Stille and Suzuki condition were performed on the same starting materials in order determine which coupling gave the best results.

2.2.1 2-Choloropurine derivatives

Compounds **18l** and **18m** (Table 2) have fluoride on the para- and meta-position on phenyl, respectively. They have been tested as selective antagonists for ARs. In order to see the effect of the fluoride position, it was decided to synthesize a molecule with orto position for the halogen with a EWD group on the purine 2-position **(27a)** (Figure 12)**.**

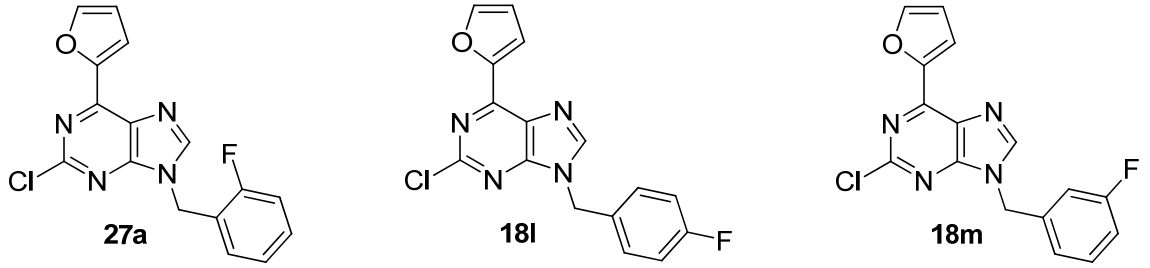
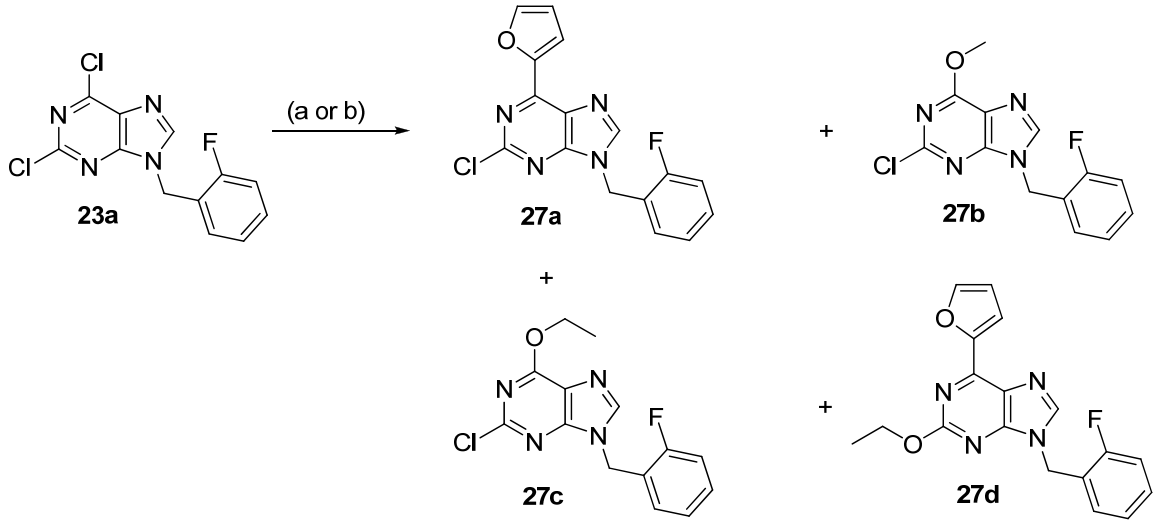


Figure 12. Compound **27a** is the target molecule.

2.2.1.1 Synthesis of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9H-purine (27a)

2,6-Dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a)** was coupled with the organostannane 2-(tributylstannyl)furan in the presence of tetrakis[tri(2-furyl)phosphine]palladium(0) in DMF at 50 ºC (Scheme 13, a).



Scheme 13. Reagents and condition: (a) Stille: 2-furylSnBu$_3$, [(2-furyl)$_3$P]$_4$Pd, DMF, 18 hours at 50 ºC; (b) Suzuki: (2-furylBF$_3$)K, K$_2$CO$_3$, (Ph$_3$P)$_2$PdCl$_2$, EtOH, 4 hours at 30-50 ºC.

Since compound **23a** has two chlorines attached, there is a possibility for coupling in the purine 2-position as well.[50] To receive a selective coupling in purine 6-posistion and the formation of the desired product **27a,** mild reaction condition must be applied. Low reaction temperature and a more reactive catalyst like [(2-furyl)$_3$P]$_4$Pd is required.[27,53] Under these conditions the dichloropurine **(23a)** reacted with almost complete region

selectivity and gave the desired coupling product **27a** in 50 % yield**.** 11 % of starting material was also recovered.

The 6-position in purine moiety will readily undergo nucleophilic aromatic substitution reactions when treated with alcohols. It has been reported that calculations have also shown that purine 6-position is more reactive towards nucleophilic attack compared to purine 2-position.[40] In the first attempt to synthesize **27a**, 6-methoxypurine[27] **27b** was observed. The ratio of the mixed compounds was 9:6 **(27a:27b)** and the products were confirmed by MS. Unfortunately they were inseparable by flash chromatography. During workup a saturated solution of KF in methanol was added in the crude mixture to convert the toxic co-product $ClSnBu_3$ to the corresponding insoluble $FSnBu_3$ (Scheme 5). In order to avoid alcohol and the formation of by-product **27b**, KF in THF was used instead. This worked successfully and gave only the desired product **27a**.

The boron reagent potassium furan-2-yltrifluoroborat was coupled with 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a)** (Scheme 13, b). Bis(triphenylphosphine)palladium (II) chloride was used as catalyst. The chlorides help to stabilize the active zerovalent Pd (0) in solution.[66] This catalyst has been utilized in a many couplings reactions previously performed in our group.[27,29-33,49-52,67] In most cases DMF was the solvent. Ethanol was used as solvent because a test reaction had been done before and the coupling had worked. In additional it is a more environmental friendly solvent compared to DMF.

The reaction was tracked on TLC while increasing the temperature. At 30 - 45 ºC no reaction took place, only when temperature was increased to 50 ºC product was seen on TLC. Reaction was stopped after four hours when no staring material was visible. During flash chromatography two fractions with different products were isolated. Both fractions had the same R_f value, they were differentiated by UV light. Initially only the product **27a** was expected to be isolated. In the first fraction 36 % of 6-ethoxypurine **(27c)** was isolated. Because of very short reaction time the staring material did not undergo nucleophilic aromatic substitution with ethanol. The second fraction contained a mixture

of the desired product **27a** and compound **27d.** The ratio was 1:7 **(27a:27d)** integrated from ^{1}H NMR and they were confirmed by MS as well. It was not possible to separate them. The chloride on the desired compound **27a** reacted with ethanol to give the side product **27d** and very little product remained. The results indicate that there is a competition for the 6-position on purine. Nucleophilic aromatic substitution is faster compared to Suzuki coupling. But after coupling, purine 2-position is also prone for substitution by ethanol. This is due to the excess of ethanol, since it is used as a solvent there is no possibility of preventing the side reactions.

The structure for 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c)** could not be 100 % confirmed based on the data available at this time. There is also a possibility that it is the other isomer with ethoxy on the 2-position and chloride on the 6-position. The carbon on purine which is bound to the ethoxy group has a shift value around 160 ppm, regardless of it is positioned on 2 or 6 carbon in the aromatic ring. In additional C-2 and C-6 will couple in a same manner to CH_2-O hydrogen. As mentioned earlier it is well known that C-6 is much more reactive towards nucleophilic aromatic substitution compared to C-2. Even though interpretation of NMR spectra suggest isomer **27c,** the data collected here is not enough to conclude which isomer it is. In order to determine the structure, compound **27c** could have been crystallized and applied to x-ray crystallography.

2.2.2 2-Aminopurine derivatives

9-(4-Chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** have previously been synthesized by Kiselgof et al as a selective antagonist for A_{2A} AR.[20] Compound **28a** was decided to synthesize in order explore the affinity for A_1, A_{2B}, A_3 receptors and also to see the effect of number of chlorides on the phenyl ring. Compound **18o** (Table 2) has two fluoride attached to the phenyl ring. In order to find out if the number of halogens affects the potency and selectivity for ARs, we chose to synthesize derivate **29a.** In both cases there is a EWG on purines 2-position (Figure 13).

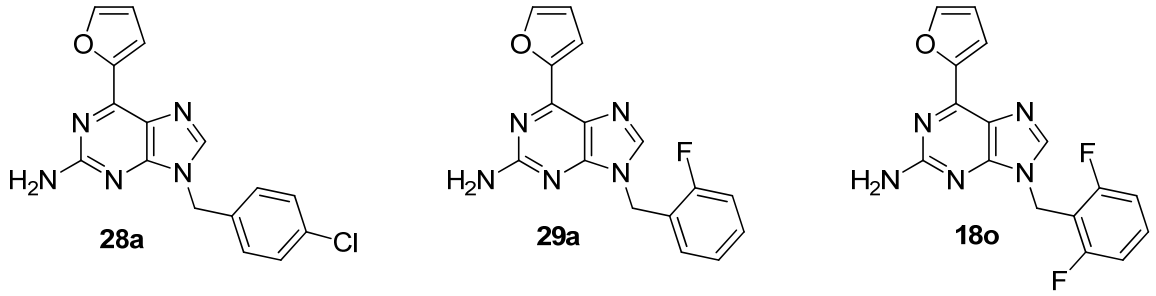
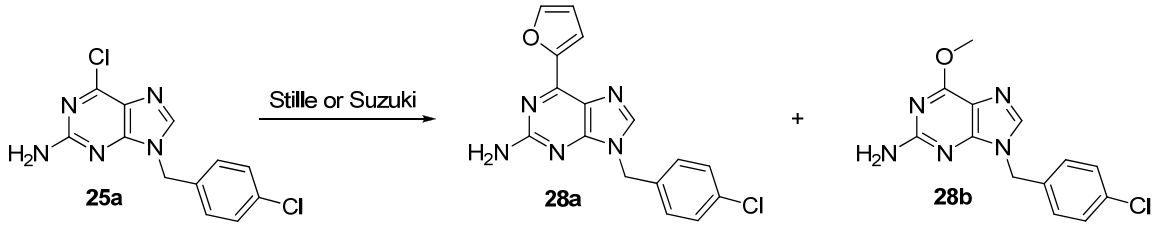


Figure 13. Compound **28a** and **29a** are the target molecules.

2.2.2.1 Synthesis of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine (28a)

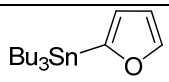
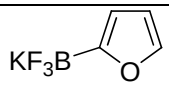
6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** was coupled using different methods in order to give 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** as product (Scheme 14).



Scheme 14. Attempted conditions used for reaction optimization is listed in Table 10: Stille coupling entry 1-4 and Suzuki coupling entry 5-6.

Table 10 gives an overview for the various methods used in Stille and Suzuki coupling to synthesize compound **28a**.

Table 10. Attempted conditions used for reaction optimization for compound **28a.**

Entry	R-(2-furyl)	Catalyst	Salt	Solvent	Temp (ºC)	Time (h)	Yield (%)
1		$(Ph_3P)_2PdCl_2$	-	DMF	90	18	61
2	"	$[(2\text{-furyl})_3P]_4Pd$	-	"	50	18	-
3	"	$[(2\text{-furyl})_3P]_4Pd$	-	"	90	18	27
4	"	$PdCl_2{\cdot}dppf{\cdot}CH_2Cl_2$	LiCl CsF	"	90	18	31
5		$Pd(OAc)_2(PPh_3)_2$	K_2CO_3	EtOH	70-80	3	50
6	"	$(Ph_3P)_2PdCl_2$	K_2CO_3	"	75	23	-

Entry 1 (Table 10) gave 61 % yield when bis(triphenylphosphine)palladium (II) chloride was used as catalyst. But when the reaction was repeated for the second time using the same conditions, the product could not be isolated. Crude ^{1}H NMR showed conversion of product. During flash chromatography a too small column was used and tin chloride adduct was mixed with product. Insufficient KF treatment (Scheme 5) caused movement of $ClSnBu_3$ on the column. Product isolated in the mixed fraction was too little to flash again. Therefore entry 1 was run for the third time. This time Ph_3PO formed by oxidation of the Ph_3P-ligand interfered with **28a.** This was proved by comparing reference ^{1}H NMR spectra for Ph_3PO. Both had same R_f value and could not be separated even though flash chromatography was done twice. In additional 20 % of starting material was also recovered.

To avoid Ph_3PO interference with product, another ligand with different polarity was chosen. Tetrakis[tri(2-furyl)phosphine]palladium(0) was the catalyst when compound **25a** reacted with 2-(tributylstannyl)furan at 50 ºC in DMF (entry 2, Table 10). Low temperature gave little formation of product. A lot of staring material which was left unreacted, reacted further with methanol during tin chloride workup (Scheme 5). These reactions gave the by product **28b**, confirmed by MS. Therefore pure product could not be isolated by flash chromatography. In order to gain better conversion of product **28a**, the temperature was raised to 90 ºC (entry 3, Table 10). Since compound **25a** has an amino group on purine 2-position it will not react with organostannane compared to staring material **23a.** Entry 3 gave full conversion of starting material **(25a)** to product, judge by ^{1}H NMR of crude mixture. The first attempt to isolate product gave a mixture with $(2\text{-furyl})_3PO$ formed by oxidation of the $(2\text{-furyl})_3P$-ligand. The main product had a slightly lower R_f value compared to $(2\text{-furyl})_3PO$. Second flash chromatography run gave 27 % of the desired product **28a.** The yield was low due to multiple separations.

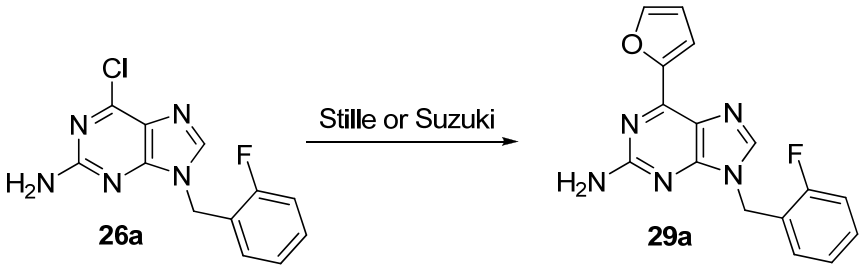
Cesium fluoride is an activator for organotin reagents in Stille coupling reactions.[68] It accelerates the transmetalation step in order to form a strong Sn-F bond, thus converting tin chloride adduct to the corresponding tin fluoride just like KF treatment (Scheme 5).[69,70] The presence of only CsF have previously been reported to convert majority of palladium catalyst to palladium black. To prevent decomposition lithium chloride was added with excess of CsF. These reactions have been reported to give high yields.[71] In order to increase the yield for compound **28a**, compound **25a** was reacted with 2-(tributylstannyl)furan employing [1,1'-Bis(diphenylphosphino)ferrocene] dichloropalladium (II)[60] as catalyst, in the presence CsF and LiCl in DMF at 90 ºC (entry 4, Table 10). But this gave only 31 % yield, even though crude TLC showed full conversion of **25a**. A reason for this could be the fact that LiCl and CsF decomposes the starting material and this results in low yield. Another reason could be that the reaction was run over night with too high temperature (90 ºC).

$Pd(OAc)_2(PPh_3)_2$[72] catalysed the Suzuki coupling between compound **25a** and potassium furan-2-yltrifluoroborat in ethanol (entry 5, Table 10). The reaction was tracked on TLC. After 1.5 hours with 70 ºC product was viewed. Temperature was increased to 80 ºC and after 3 hours the reaction was stopped when no staring material was visible on TLC. The resulting crude mixture was purified by dry flash chromatography on silica gel using ethanol and filtrated with a mixture of hexane/ethanol/cold water. The product precipitated when a combination of Ethyl acetate/hexane was added. A new and less time consuming technique was developed herein without the use of ordinary flash chromatography. This gave 50 % of the desired product **28a.**

To increase the yield compared to entry 5 (Table 10), entry 6 was run over night with a set temperature at 75 ºC. Three different compounds were present in the crude mixture, judged by ^{1}H NMR. After flash chromatography a mixture of compound **25a, 28a** and **28b** was isolated in a ratio of 2:5:3 respectively. They were confirmed by MS as well. Pure products could not be isolated due to insufficient conversion of starting material. Suzuki coupling had taken place rather quickly and when the catalyst decomposed, the remaining starting material reacted with ethanol and by product **28b** was formed. Compound **28b** was not observed in entry 5. It seems like longer reaction time trigger formation of by product and the temperature should have been 80 ºC instead, in order to increase coupling speed.

2.2.2.2 Synthesis of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine (29a)

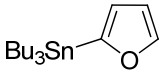
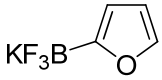
Organostannane 2-(tributylstannyl)furan and the boron reagent potassium furan-2-yltrifluoroborat were Stille- or Suzuki coupled with 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** employing different method in order to give compound **29a** (Scheme 15).



Scheme 15. Attempted conditions used for reaction optimization is listed in Table 11: Stille coupling entry 1-4 and Suzuki coupling entry 5-6.

Table 11 gives a detailed overview of how the synthesis of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** was done.

Table 11. Attempted conditions used for reaction optimization for compound **29a.**

Entry	R-(2-furyl)	Catalyst	Salt	Solvent	Temp (ºC)	Time (h)	Yield (%)
1		$(Ph_3P)_2PdCl_2$	-	DMF	90	18	-
2	"	$[(2\text{-furyl})_3P]_4Pd$	-	"	90	18	-
3	"	$PdCl_2{\cdot}dppf{\cdot}CH_2Cl_2$	-	"	90	18	-
4	"	$PdCl_2{\cdot}dppf{\cdot}CH_2Cl_2$	LiCl CsF	"	90	18	29
5		$Pd(OAc)_2(PPh_3)_2$	K_2CO_3	EtOH	75-85	4	83
6	"	$(Ph_3P)_2PdCl_2$	K_2CO_3	"	80	4	72

Again oxidization of ligand to Ph_3PO gave problems during flash chromatograpgy for compound **29a** as well (entry 1, Table 11). Pure product could not be isolated with ethyl acetate/ hexane, although no starting material could be seen from ^1H NMR of crude mixture. The catalyst was changed to $[(2\text{-furyl})_3P]_4Pd$ in order to give different R_f value for product and oxidized ligand (entry 2, Table 11). Crude NMR showed full conversion of starting material. But flash chromatography gave many challenges. A too small column was used, insufficient KF treatment (Scheme 5) resulted in movement of tin chloride adduct on the column and the product was not able to move on the column due to the use of less polar solvent system (ethyl acetate/ hexane). Only 20 mg of compound **29a** was isolated, but it was contaminated with tin chloride adduct. To get a better separation, entry 2 (Table 11) was run for the second time. And again crude ^1H NMR showed full conversion of starting material. The reaction condition is very good in term of reactivity. Different solvent systems were tried on TLC. When a combination of DCM/EtOH was employed during flash chromatography it gave good separation and 450 mg (73 %) of **29a** was isolated. Unfortunately some tin chloride adduct was also present because KF treatment did not work well. Apart from that, no other contaminations were present. See Figure 14 for ^1H NMR.

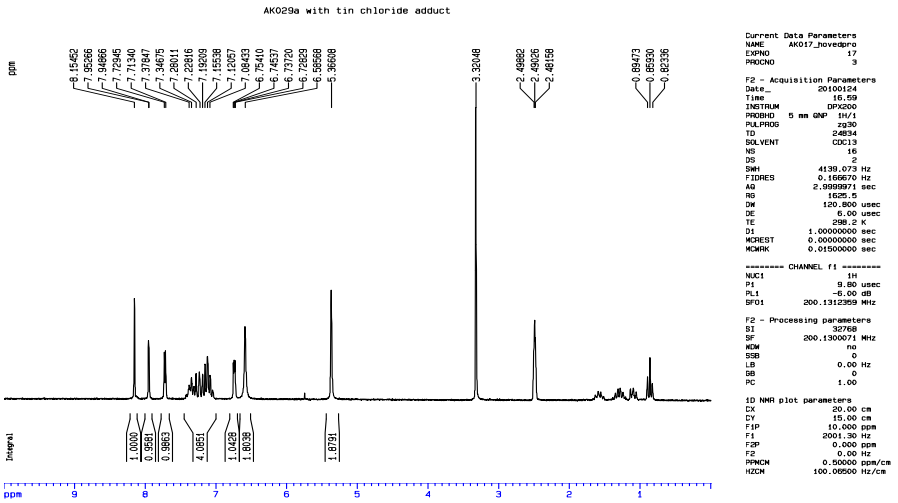


Figure 14. ^1H NMR of isolated compound **29a** contaminated with $ClSnBu_3$ in 1.8-0.8 ppm region**. (**Entry 2, Table 11).

Acetonitrile was added to the isolated compound in order to remove $ClSnBu_3$.[33] But compound **29a** was insoluble. Therefore a saturated solution of KF in THF was added and a second flash chromatography was performed with chloroform/MeOH. But almost all product got stuck on the column and could not be isolated again.

6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** was coupled with 2-(tributylstannyl)furan. [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium (II) was the catalyst (entry 3, Table 11). Crude NMR proved that Stille coupling had taken place. But little product was isolated (< 20 mg) and it also contained oxidized ligand. The same condition was applied once more, but this time CsF and LiCl were added (entry 4, Table 11). This gave only 29 % of product **29a**. Similarly for compound **28a** with same condition (entry 4, Table 10), this also resulted in low yield. This verifies that with the salts involved, the starting material decomposes. The temperature and reaction time should also be decreased.

Suzuki conditions were also explored with 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a).** In the fifth entry (table 11) $Pd(OAc)_2(PPh_3)_2$ was the catalyst. Palladium (II) acetate has previously been reported to be successful in Suzuki coupling of boron reagent.[60] The reaction was followed by TLC. After 1 hour at 75 ºC product formed. Temperature was increased to 85 ºC after 3.5 hours and stopped in the 4th hour when no starting material **(26a)** was visible on TLC. Product precipitated in the solvent during reaction. Filtration with water/hexane/ethanol/methanol gave 83 % yield. The new method for purification does not require ordinary flash chromatography and is the highest yield obtained for coupling reaction compared to the other entries (Table 10 and 11).

In order to see how $(Ph_3P)_2PdCl_2$ works as catalyst for Suzuki coupling for compound **26a**, entry 6 (Table 11) was run. The temperature was set to 80 ºC, based on reaction from entry 5 (Table 11). After 4 hours full conversion of starting material was achieved.

Flash chromatography with DCM/MeOH and crystallization with DCM/chloroform gave 72 % yield. This is also a very good yield, indeed. No other by product was formed with the use of ethanol as solvent. This confirms that short and effective reaction gives only the desired product with 2-aminopurine derivatives (Scheme 14 and 15). For general reaction mechanism for Suzuki coupling, see Scheme 7.

2.3 Acetylation of amine

Compound **18q** and **18s**, with mono- and diacetylated amine have been tested as selective antagonist for the ARs (Table 2, Figure 16). This is a new line of derivatives compound **18**, developed in our group. In order to see the quantitative and qualitative effect of halogen on the aromatic ring and the significance of mono- and diacetamide on purine 2-position, 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** and 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** were further subjected acetylation. Target molecules are presented in Figure 16.

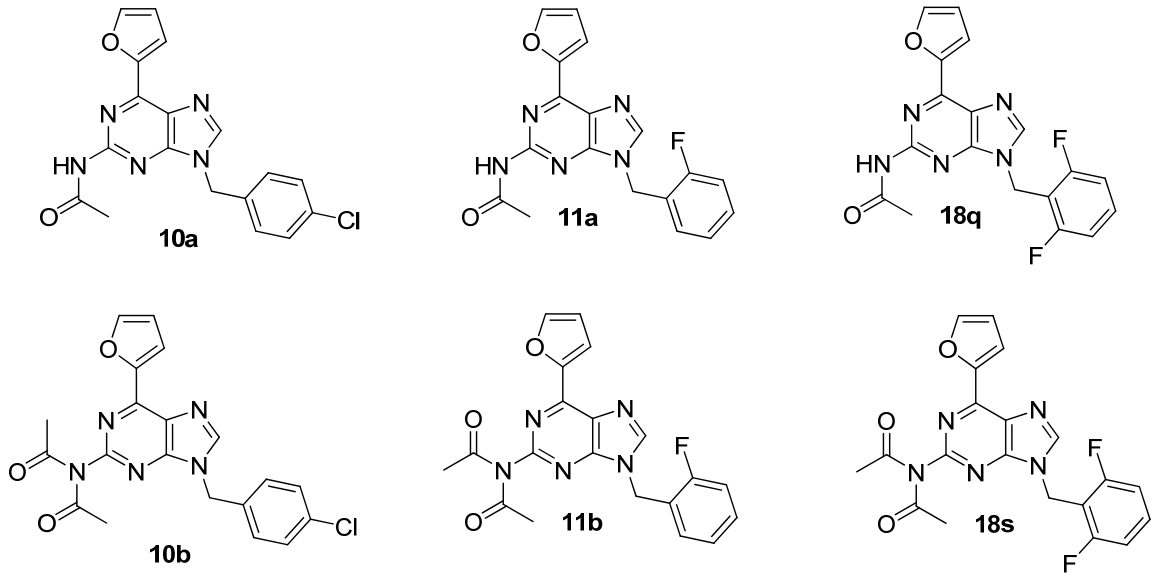
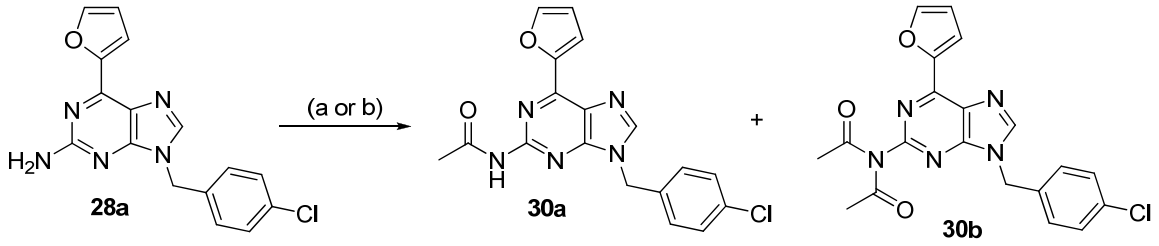


Figure 16. Compounds **10a, 10b, 11a** and **11b** are the target molecules.

2.3.1 Synthesis of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide (30a) and *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide (30b)

9-(4-Chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** was refluxed for 24 hours with acetic anhydride and triethylamine in toluene (Scheme 17, a). For reaction mechanism see Scheme 9.

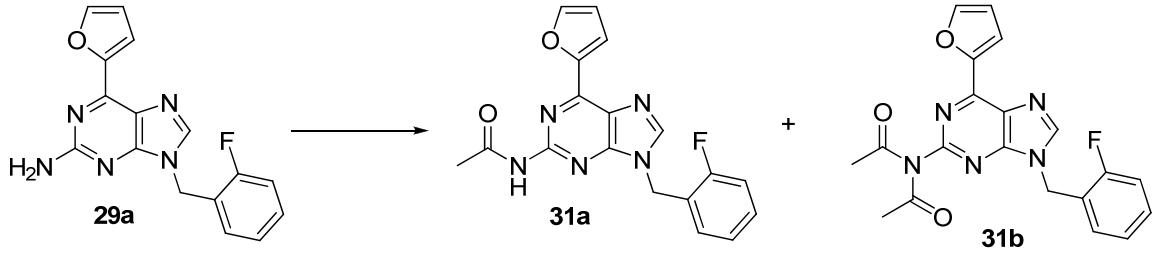


Scheme 17. Reagents and condition: (a) Ac_2O, Et_3N, Toluene, 24 hours of reflux; (b) Ac_2O, Toluene, 48 hours of reflux.

This gave the monoacetylated compound **30a** in 53 % yield and diacetylated **(30b)** in 9 % yield. Compound **30b** was only formed in minor amount when base was employed (Scheme 9).[32,61] When base was not added (Scheme 17, b)[32,62] *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)** was formed in 66 % yield after 48 hour reflux. Less than 7 % of **30a** was also formed, but it was mixed with a small amount of **30b.** It has been proved herein that the selectivity is reversed and it is possible to control the quantity when base is added or removed. The compounds were separated by flash chromatography using ethyl acetate/ hexane.

2.3.2 *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl) acetamide (31a) and *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl) acetamide (31b)

9-(4-Fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** was reacted with the conditions described in Scheme 18 to give *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)** and *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b).**



Scheme 18. Attempted conditions used for reaction optimization is listed in Table 12.

Table 12. Attempted conditions used for reaction optimization for compounds **31a** and **31b.**

Entry	31a (%)	31b (%)	Reagent	Base	Solvent	Time (h)
1	63	-	Ac_2O	Et_3N	Toluene	24
2	4	-	″	Et_3N	″	24
3	59	4	″	-	″	48
4	21	32	″	-	″	24

Entry 1[32,61] (Table 12) gave 63 % of compound **31a**, when refluxed for 24 hours. Very minor amount of product **31b** was isolated, but it was mixed with the monoacetylated compound **(31a).** It was only 10 mg. In order to synthesize more of compound **31b**, the reaction was repeated (entry 2, Table 12). But this gave only 4 % of **31a,** due to poor crystallization during work up. The base was removed (entry 3, Table 12)[32,62,63] in order to achieve better selectivity of diacetylated compound like the one in Scheme 17, b. Surprisingly the selectivity was reversed and the monoacetylated was formed in major amount. In order to explore the effect of reaction time, entry 4 (Table 12) was run only for 24 hours. This shifted the ratio and the diacetylated compound **(31b)** was isolated in major amount by flash chromatography.

3. CONCLUSION

All the seven target molecules (**27a, 28a, 29a, 30a, 30b, 31a** and **31b**) were successfully synthesized (Figure 17). They have been sent for biological testing for the various adenosine receptors, in order to see if they can be utilized as potential drugs in the future. The screening is carried out in the group of Professor Christa Müller at the University of Bonn.

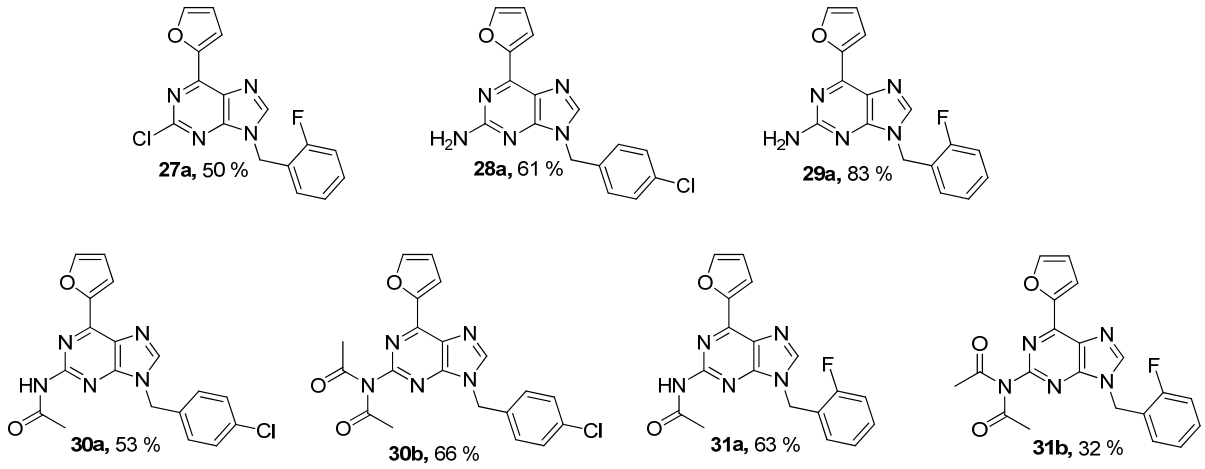


Figure 17. Target compounds presented with yields.

For compound **23a** with chloride on the 2-position Stille coupling worked better. By doing some optimization for the Suzuki coupling it is still possible to achieve pure product **(27a)**. In this case the solvent must be non-nucleophilic to prevent side reaction with chlorides on the purine moiety. The temperature should be at 50 ºC and the other reagents can be kept as they were.

Ethanol as solvent can be employed in Suzuki coupling for 2-aminopurines when the reaction time is short, but over night reactions produce by product and different solvent should be applied. Suzuki coupling works better for compound **25a,** compared to Stille coupling. Suzuki coupling has been proved to be more efficient. Compared to Stille reactions, it has been additionally confirmed herein that Suzuki coupling gives highest yields, less time consuming reactions, less unhealthy, much easier purification, produce no toxic by products and most environmental friendly.

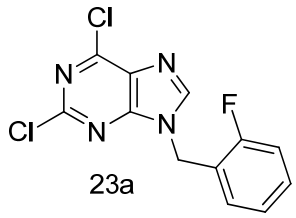
4. EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 200 MHz a Bruker DPX 200 instrument or at 300 MHz with a Bruker DPX 300 instrument. The ^{13}C NMR spectra* were recorded at 75 or 50 MHz using the above mentioned instruments. Peak assignment in ^{1}H NMR and ^{13}C NMR was based on information obtained from HMQC and HMBC spectroscopy. Mass spectra were recorded on a VG Prospec sector instrument from Fission Instrument at 70 eV ionizing voltage and are presented as *m/z* (% rel. int.). Melting points were determined with a Büchi melting point B-545 apparatus and are uncorrected. Flash chromatography was done manually with silica gel from Merck (60, 40-63 μm).

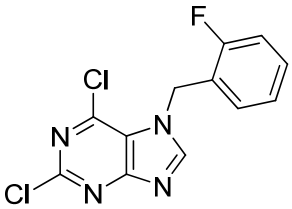
DMF and THF were taken dry from MBRAUN Solvent Purification System (SPS). Ethylacetate, hexane, dichloromethane, acetic anhydride, triethylamine and toluene were purified by distillation. Compound **AN18a** and potassium furan-2-yltrifluoroborat were previously synthesized in the group. All other reagents were commercially available and used as received. Elemental analyses were performed by Lianne Hill, School of Chemistry, University of Birmingham, England.

* In all ^{13}C NMR spectra (except for compound **27a**) there is a peak present at ca 79 ppm. This is due to noise from the NMR instrument.

2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine (23a) and 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine (23b)



23a



23b

A mixture of 2,6-dichloropurine **(22a)** (944 mg, 5.00 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in dry DMF (20 mL) was stirred at r.t. under N_2 for 30 min., before 2-fluorobenzyl chloride (1.1 mL, 9.0 mmol) was added. The resulting mixture was stirred under r.t for 18 h, filtered and evaporated *in vacuo*. The products were purified by flash chromatography on silica gel using ethyl acetate/hexane (1:1) followed by ethyl acetate. This gave 900 mg (61 %) of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a)** as a colourless powder and 280 mg (19 %) of 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b)** as a colourless powder.

2,6-Dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a)**

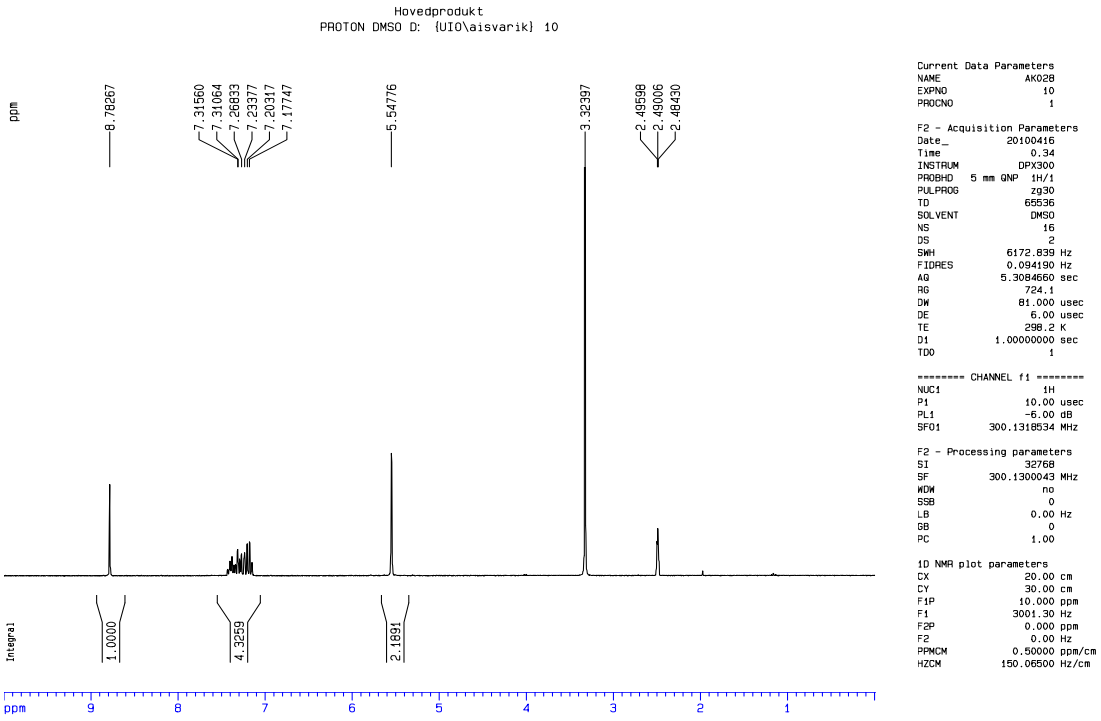
^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.79 (s, 1H, H-8), 7.44-7.16 (m, 4H in Ar), 5.57 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 159.9 (d, J_{CF} = 246 Hz, CF in Ar), 153.3 (C-4), 151.0 (C-6), 149.7 (C-2), 148.3 (C-8), 130.5 (d, J_{CF} = 8.2 Hz, CH in Ar), 130.4 (C-5), 130.1 (d, J_{CF} = 3.8 Hz, CH in Ar), 124.7 (d, J_{CF} = 3.5 Hz, CH in Ar), 122.3 (d, J_{CF} = 14.5 Hz, C in Ar), 115.5 (d, J_{CF} = 20.8 Hz, CH in Ar), 41.4 (CH_2).

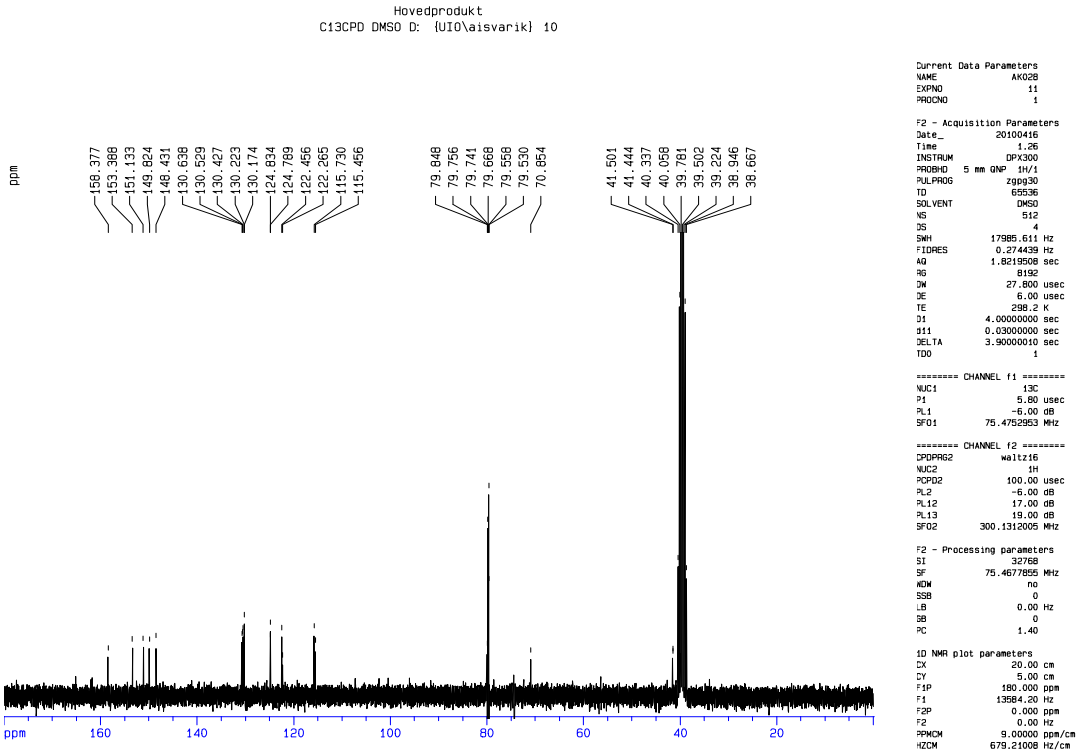
MS EI *m/z* (rel. %): 300/298/296 (5/27/40, M^+), 109 (100), 83 (23).

HR-MS Found 296.0029, calcd. for $C_{12}H_7Cl_2FN_4$ 296.0032.

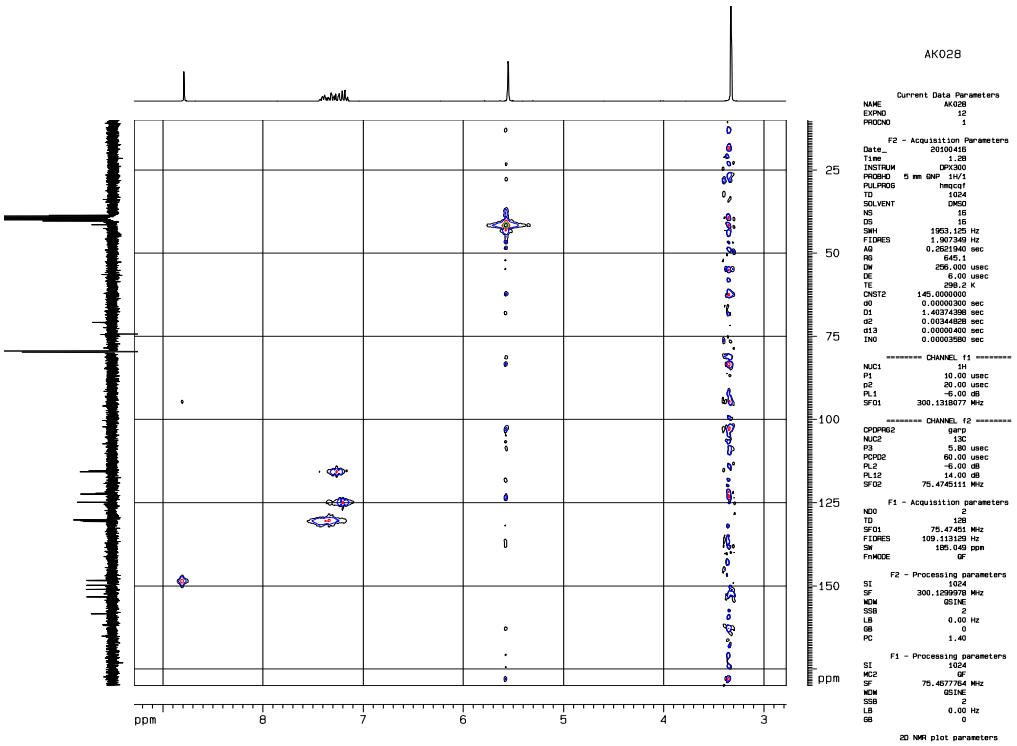
M.p. 172 ºC



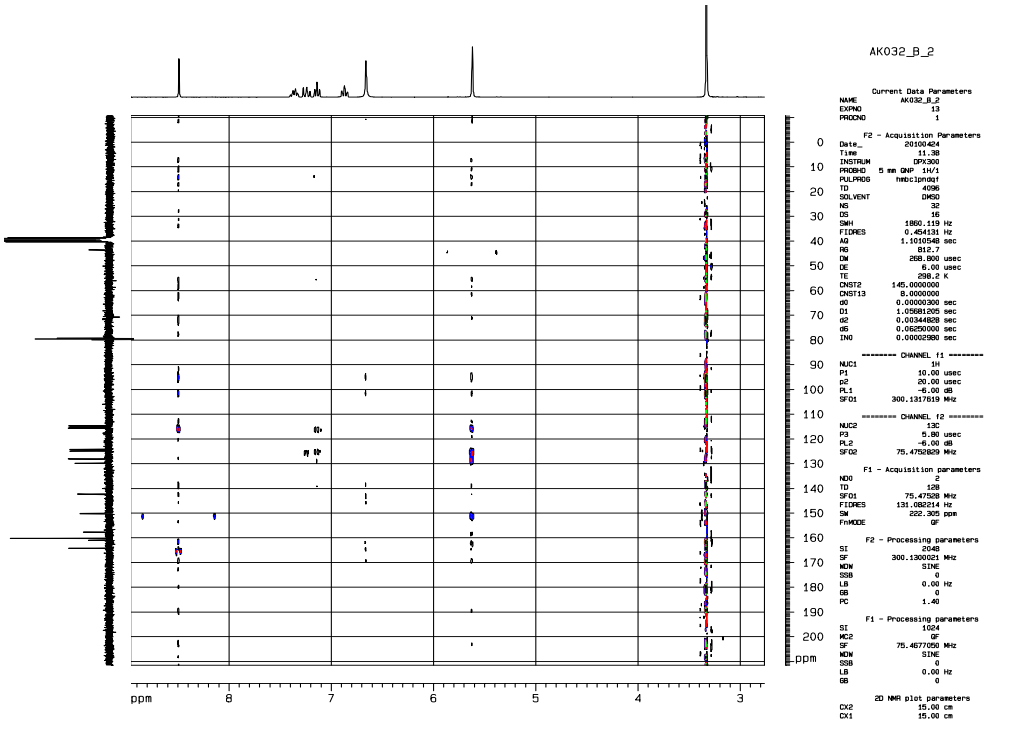
Spectrum 1. [1]H of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a).**



Spectrum 2. [13]C of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a).**



Spectrum 3. HMQC of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a).**



Spectrum 4. HMBC of 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a).**

2,6-Dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b)**

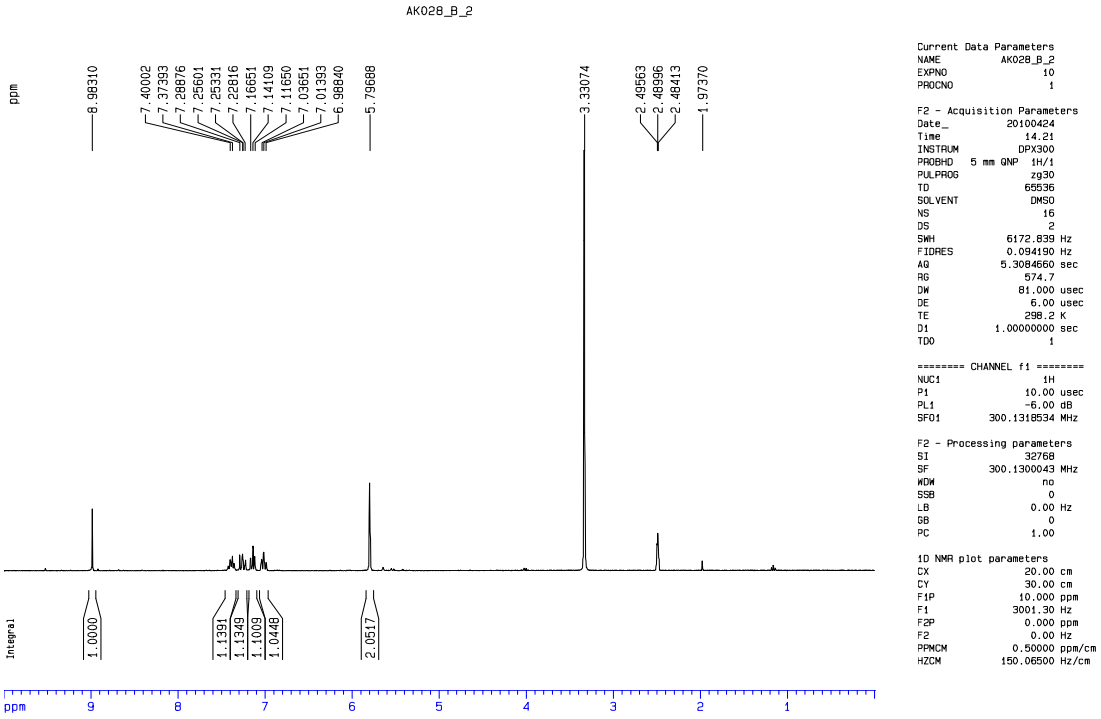
^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.00 (s, 1H, H-8), 7.44-7.00 (m, 4H in Ar), 5.81 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 163.3 (C-4), 159.3 (d, J_{CF} = 246 Hz, CF in Ar), 153.1 (C-8), 151.2 (C-2), 143.1 (C-6), 130.2 (d, J_{CF} = 8.3 Hz, CH in Ar), 128.5 (d, J_{CF} = 3.5 Hz, CH in Ar), 124.9 (d, J_{CF} = 3.5 Hz, CH in Ar), 123.4 (d, J_{CF} = 13.9 Hz, C in Ar), 121.9 (C-5), 115.4 (d, J_{CF} = 20.7 Hz, CH in Ar), 44.2 (CH_2).

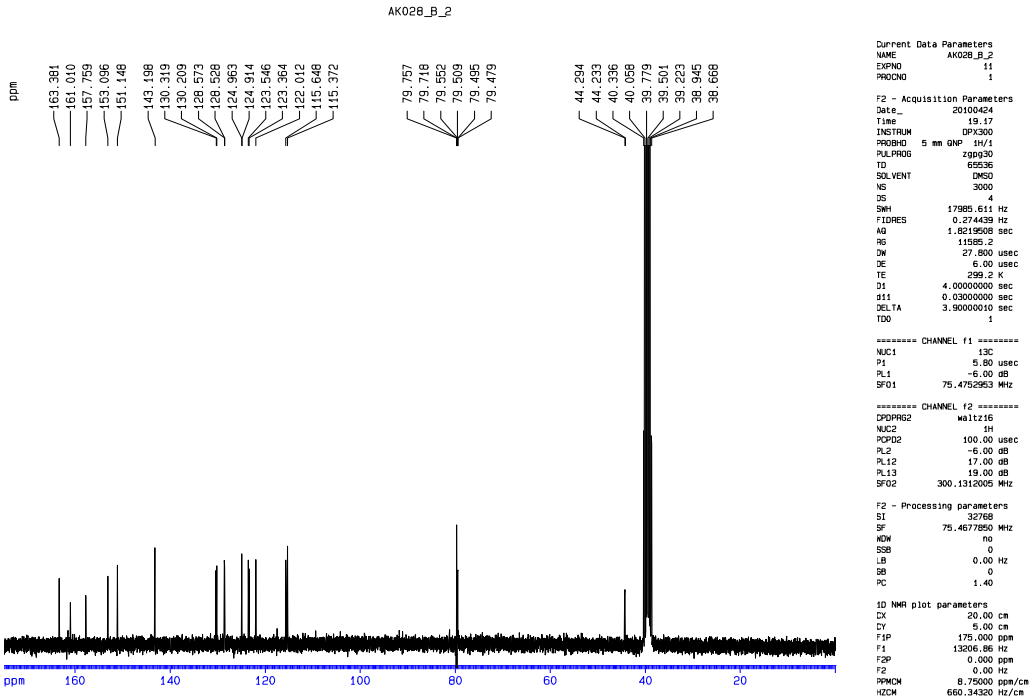
MS EI *m/z* (rel. %): 300/298/296 (3/19/28, M^+), 109 (100), 83 (15).

HR-MS Found 296.0034, calcd. for $C_{12}H_7Cl_2FN_4$ 296.0032.

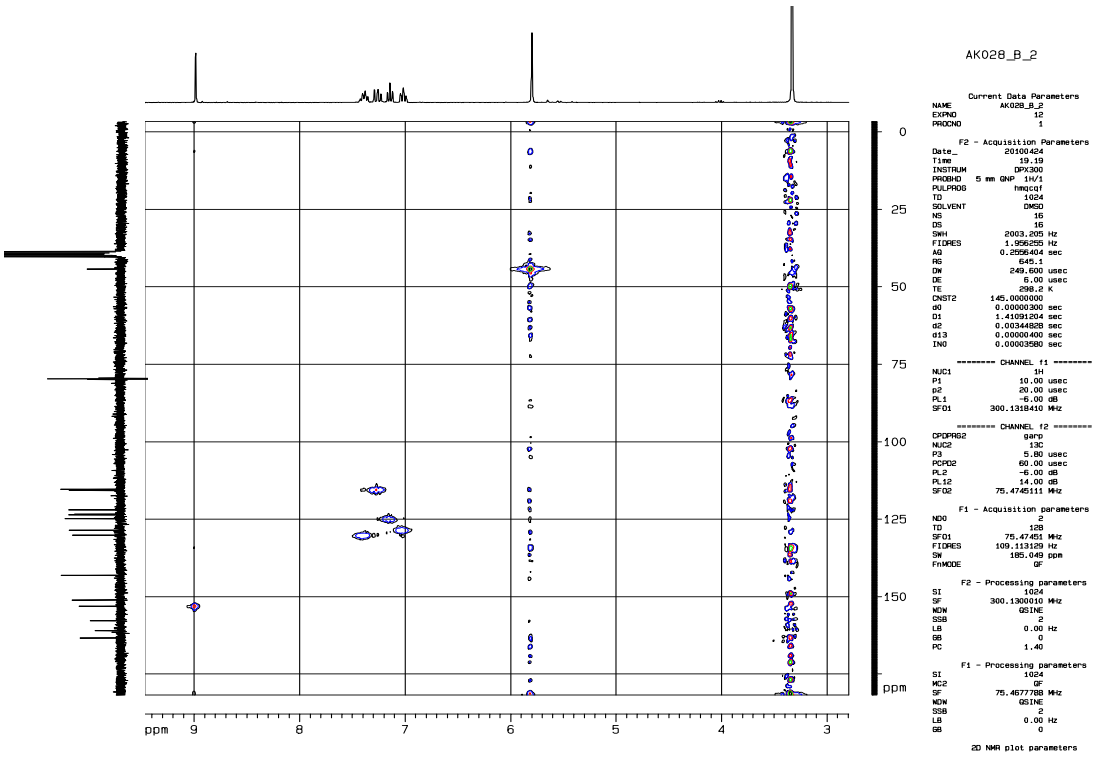
M.p. 191 ºC



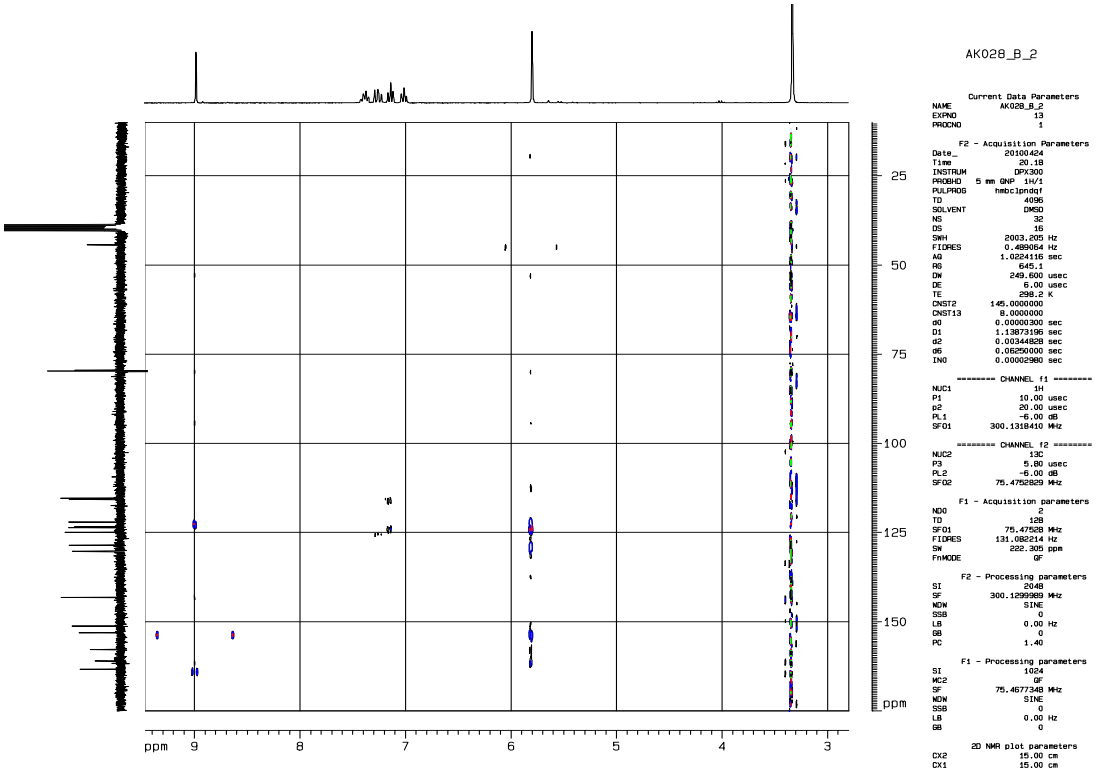
Spectrum 5. ^{1}H of 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b).**



Spectrum 6. ^{13}C of 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b).**

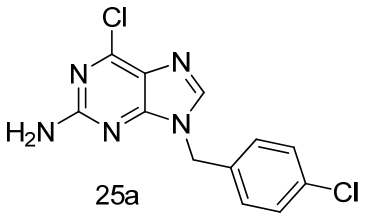


Spectrum 7. HMQC of 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b).**

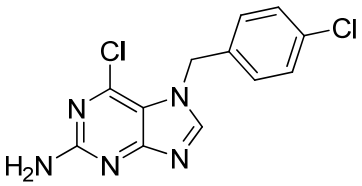


Spectrum 8. HMBC of 2,6-dichloro-7-(2-fluorobenzyl)-7*H*-purine **(23b).**

Synthesis of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine (25a) and 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine (25b)



25a



25b

A mixture of 2-amino-6-chloropurine **(24a)** (846 mg, 5.00 mmol) and K_2CO_3 (2.06 g, 15.0 mmol) in DMF (20 mL) was stirred at r.t. under N_2 for 1 h. A solution of 4-chlorobenzyl chloride (1.6 g, 0.01 mmol) in DMF (4 mL) was added and the resulting mixture was stirred at r.t for 23 h. The mixture was filtrated and evaporated *in vacuo.* The products were purified by flash chromatography on silica gel using different solvent systems (Table 13). This gave 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** as a colourless fluffy powder and 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b)** as a yellow powder. Amounts and yields are given in Table 13.

Table 13. Optimization of reaction conditions.

Entry	**Compound**	**Amount (mg)**	**Yield (%)**	**Flash chromatography Solvent system**
1	25a	268	18	EtOAc/Hexane (2:1)
	25b	-	-	
2	25a	437	30	EtOAc/Hexane (2:1) then (1:0)
	25b	-	-	
3	25a	930	63	EtOAc/Hexane (2:1) then (1:0)
	25b	-	-	
4	25a	190	13	DCM/MeOH (95:5)
	25b	-	-	
5	25a	770	52	DCM/MeOH (98:2) then (94:6)
	25b	202	15	
6	25a	910	62	DCM/MeOH (98:2) then (90:10)
	25b	200	14	

6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)**

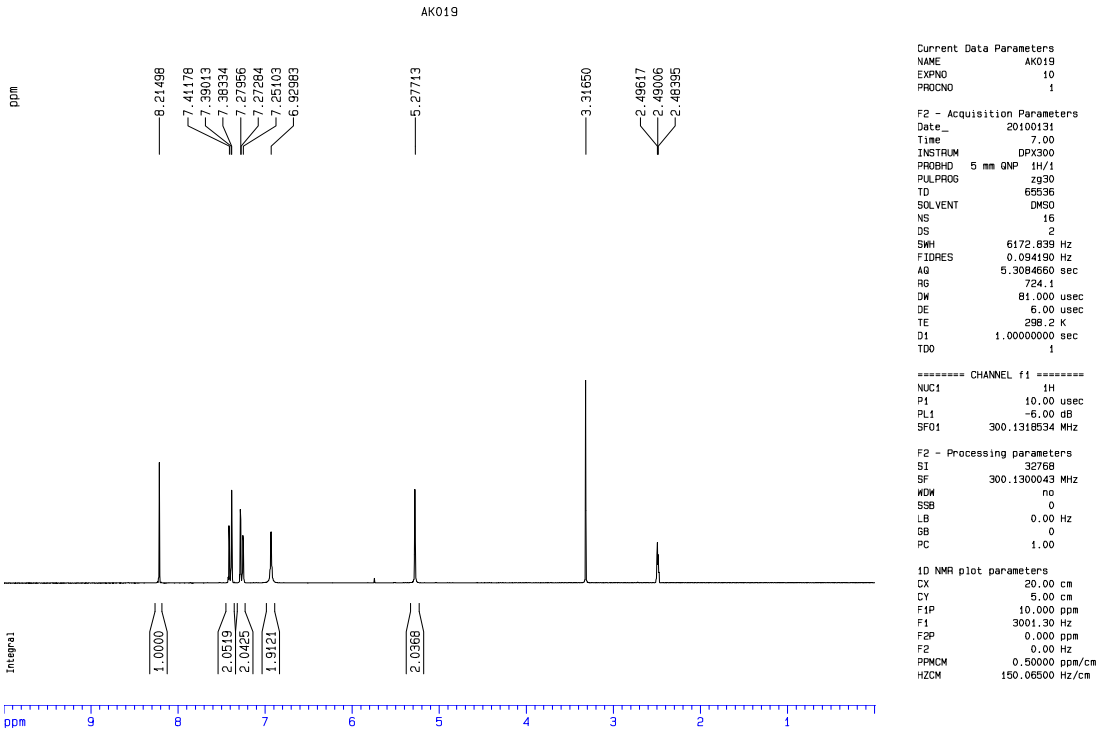
^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.21 (s, 1H, H-8), 7.40 (d, J = 7.3 Hz, 2H in Ar), 7.28 (d, J = 7.3 Hz, 2H in Ar), 6.93 (br s, 2H, NH_2), 5.28 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 159.9 (C-6), 154.0 (C-4), 149.5 (C-2), 143.1 (C-8), 135.6 (C in Ar), 132.4 (C-4 in Ar), 129.1 (CH in Ar), 128.7 (CH in Ar), 123.3 (C-5), 45.4 (CH_2).

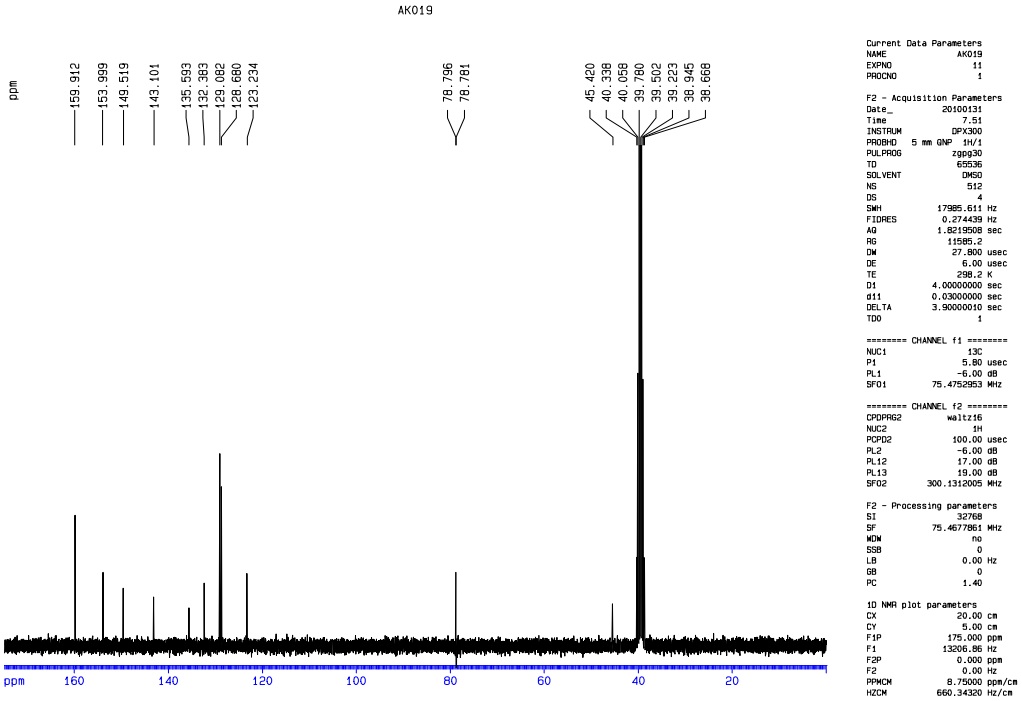
MS EI *m/z* (rel. %): 297/295/293 (5/33/51, M^+), 127/125 (33/100), 89 (11).

HR-MS Found 293.0235, calcd. for $C_{12}H_9Cl_2N_5$ 293.0235.

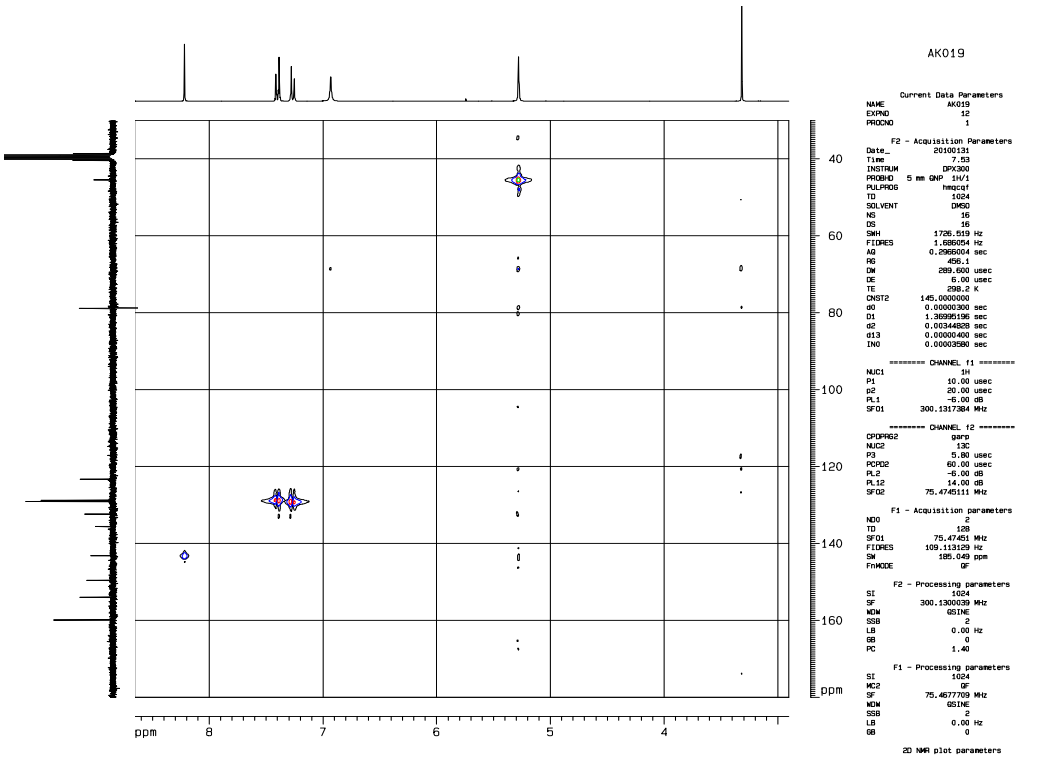
M.p. 212 ºC



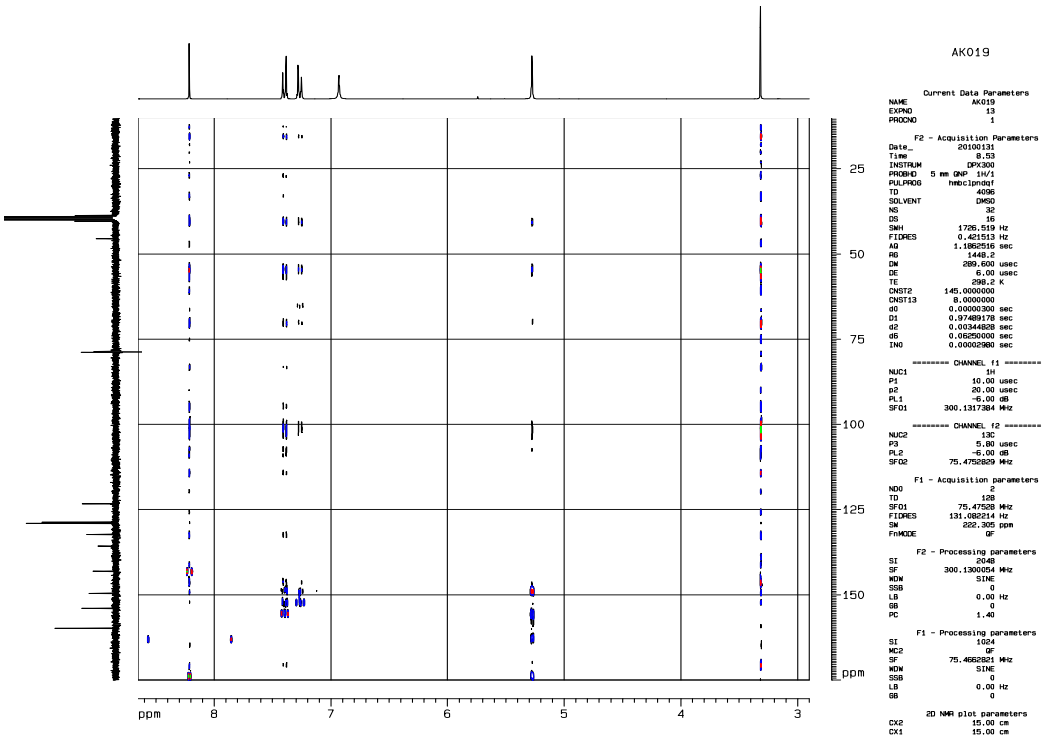
Spectrum 9. ^{1}H of 6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)**



Spectrum 10. ^{13}C of 6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a).**



Spectrum 11. HMQC of 6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a).**



Spectrum 12. HMBC of 6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a).**

6-Chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b)**

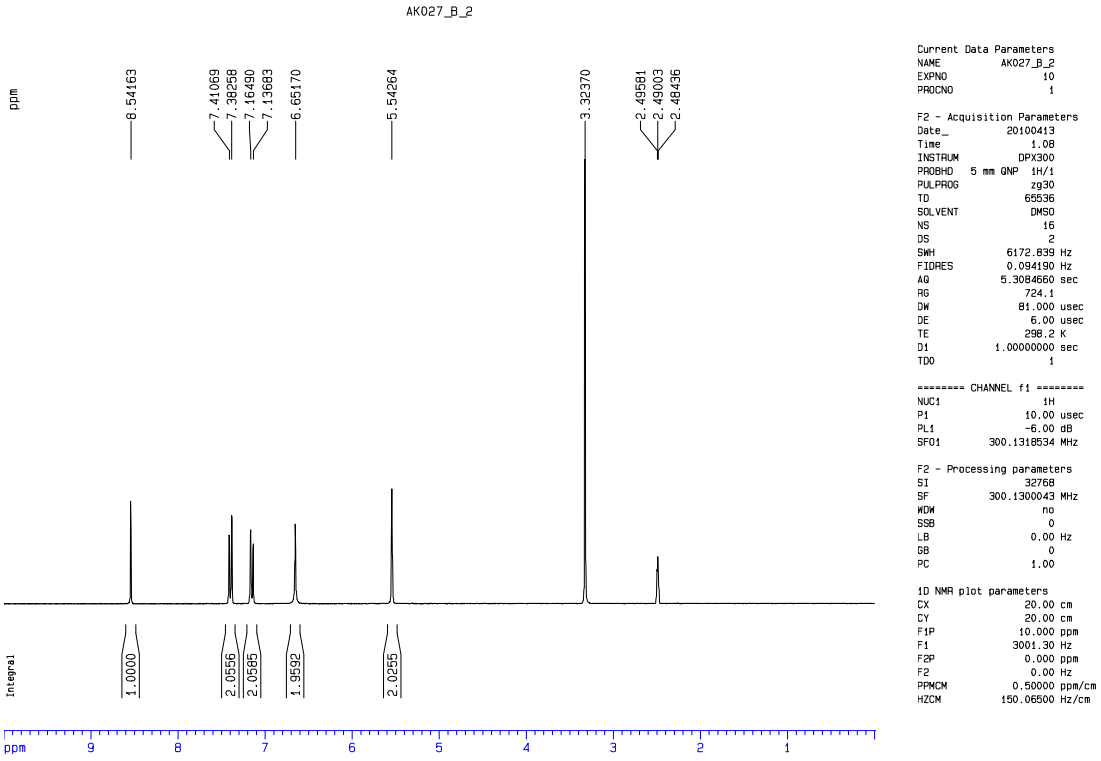
^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.55 (s, 1H, H-8), 7.41 (d, J = 9.0 Hz, 2H in Ar), 7.16 (d, J = 9.0 Hz, 2H in Ar), 6.67 (br s, 2H, NH_2), 5.55 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 164.3 (C-2), 160.0 (C-6), 149.8 (C-8), 142.2 (C-4), 136.1 (C in Ar), 132.2 (C-4 in Ar), 128.6 (CH in Ar), 128.2 (CH in Ar), 114.6 (C-5), 48.4 (CH_2).

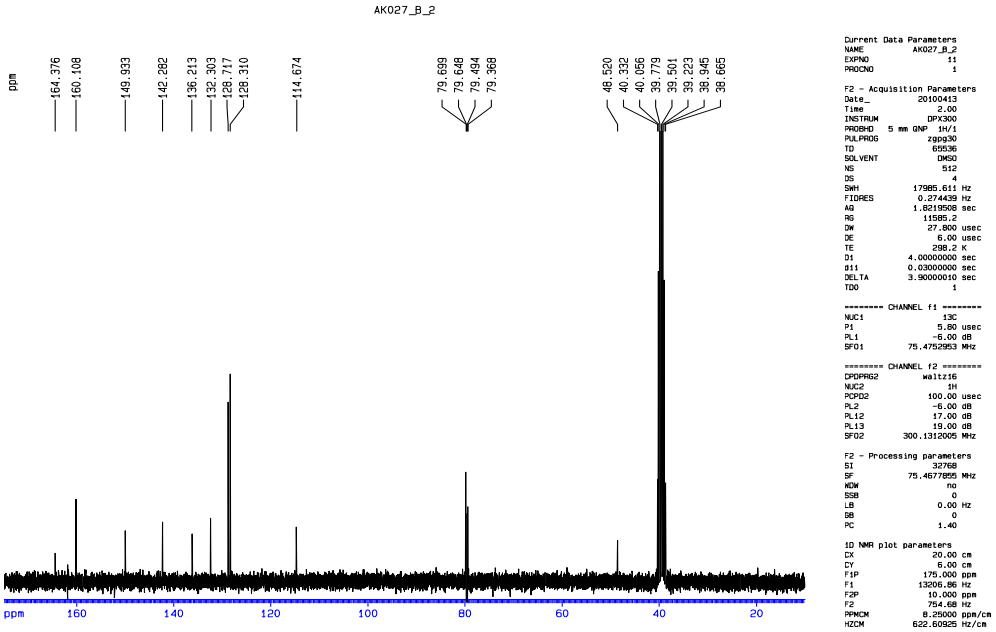
MS EI *m/z* (rel. %): 297/295/293 (4/25/38, M^+), 127/125 (33/100), 89 (11).

HR-MS Found 293.0234, calcd. for $C_{12}H_9Cl_2N_5$ 293.0235.

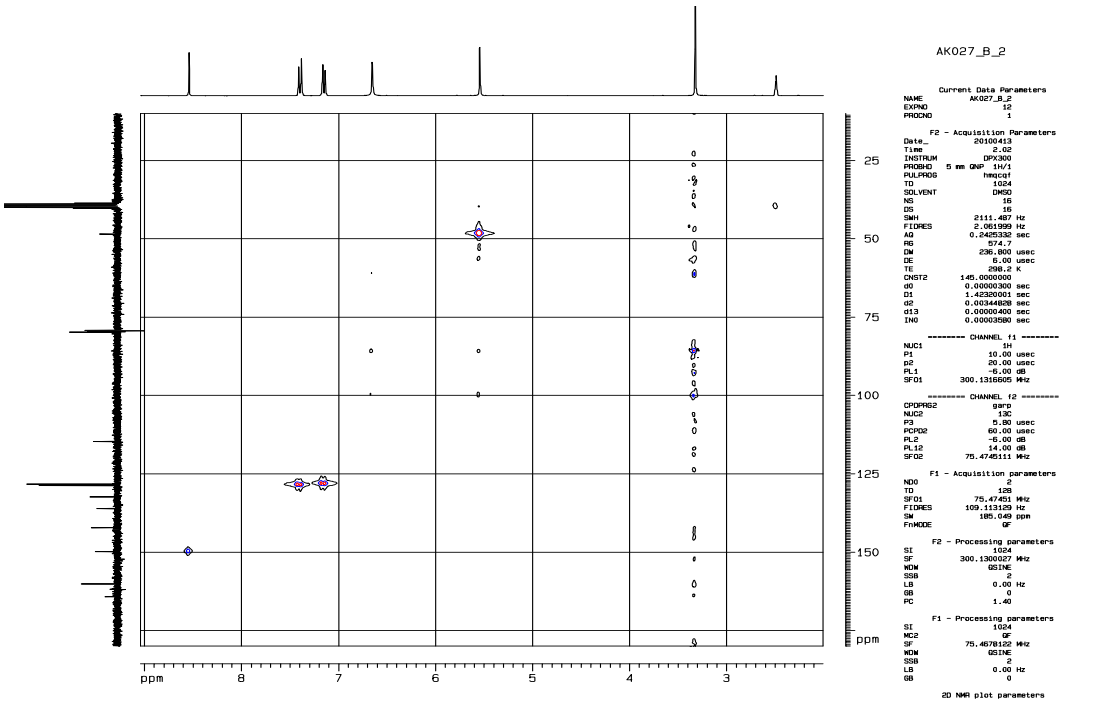
M.p. 222 ºC



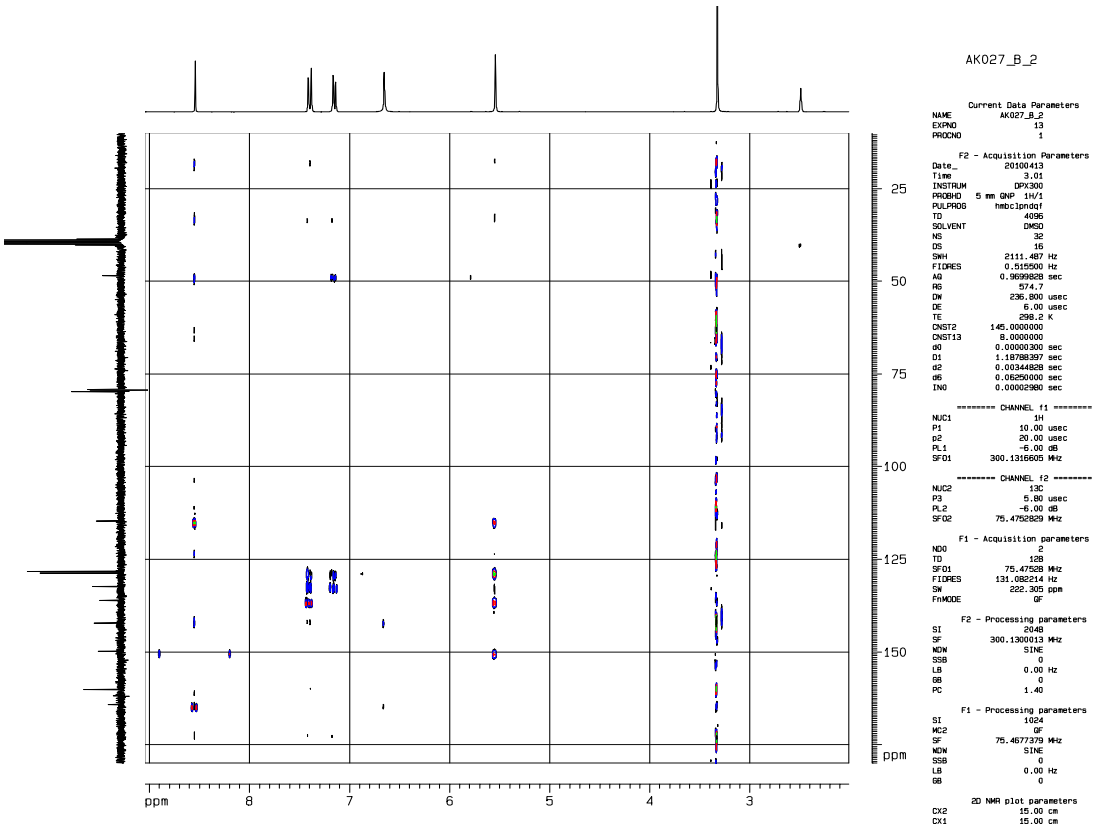
Spectrum 13. [1]H of 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b).**



Spectrum 14. [13]C of 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b).**

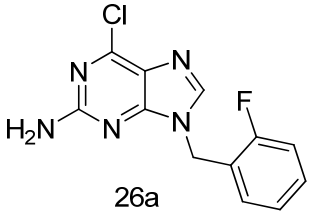


Spectrum 15. HMQC of 6-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b).**

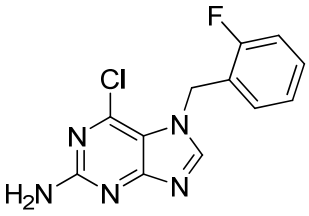


Spectrum 16. HMBC of **6**-chloro-7-(4-chlorobenzyl)-7*H*-purin-2-amine **(25b).**

Synthesis of 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine (26a) and 6-chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine (26b)



26a



26b

A mixture of 2-amino-6-chloropurin **(24a)** (845 mg, 5.00 mmol) and K_2CO_3 (2.07 g, 15.0 mmol) in dry DMF (20 mL) was stirred at r.t. under N_2 for 30 min., before 2-fluorobenzyl chloride (1.20 mL, 10.0 mmol) was added. The resulting mixture was stirred under r.t for 18 h, filtered and evaporated *in vacuo*. The products were purified by flash chromatography on silica gel using different solvent systems (Table 14). This gave 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** as a yellow fluffy powder and of 6-chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b)** as a colourless powder. Amounts and yields are given in Table 14.

Table 14. Optimization of reaction conditions.

Entry	**Compound**	**Amount (mg)**	**Yield (%)**	**Flash chromatography Solvent system**
1	26a	836	60	DCM/hexane/EtOAc (1:1:2) then (0:0:1)
	26b	-	-	
2	26a	706	51	DCM/MeOH (95:5) then (90:10)
	26b	64	5	
3	26a	780	56	DCM/MeOH (95:5) then (90:10)
	26b	30	2	
4	26a	940	68	DCM/MeOH (95:5) then (90:10)
	26b	80	6	
5	26a	960	69	DCM/MeOH (95:5) then (90:10)
	26b	100	7	

6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)**

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.14 (s, 1H, H-8), 7.40-7.33 (m, 1H, H-3 in Ar), 7.26-7.07 (m, 3H, H-2, H-4 and H-5 in Ar), 6.92 (br s, 2H, NH_2), 5.34 (s, 2H, CH_2).

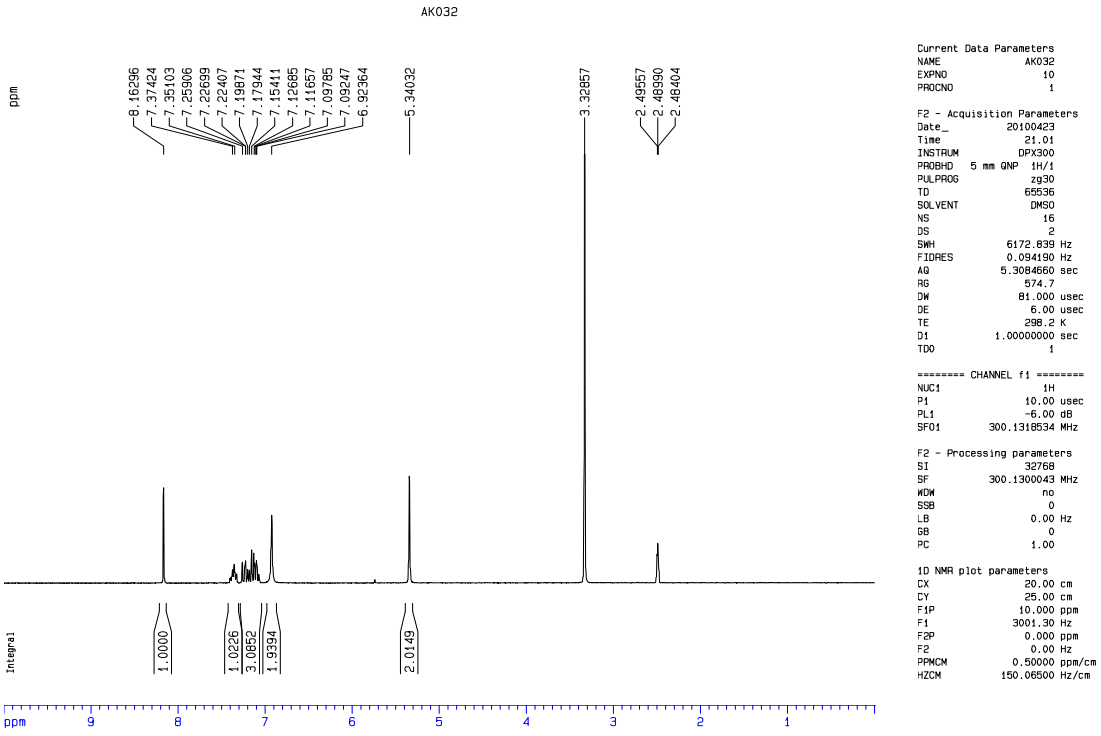
^{13}C NMR (DMSO-d_6, 75 MHz): δ 159.9 (C-2), 159.7 (d, J_{CF} = 245 Hz, CF in Ar), 154.1 (C-4), 149.5 (C-6), 143.2 (C-8), 130.1 (d, J_{CF} = 8.3 Hz, CH in Ar), 129.3 (d, J_{CF} = 3.8 Hz,

CH in Ar), 124.8 (d, J_{CF} = 3.5 Hz, CH in Ar), 123.4 (C-5), 123.2 (d, J_{CF} = 14.5 Hz, C in Ar), 115.5 (d, J_{CF} = 20.7 Hz, CH in Ar), 40.1 (CH_2).

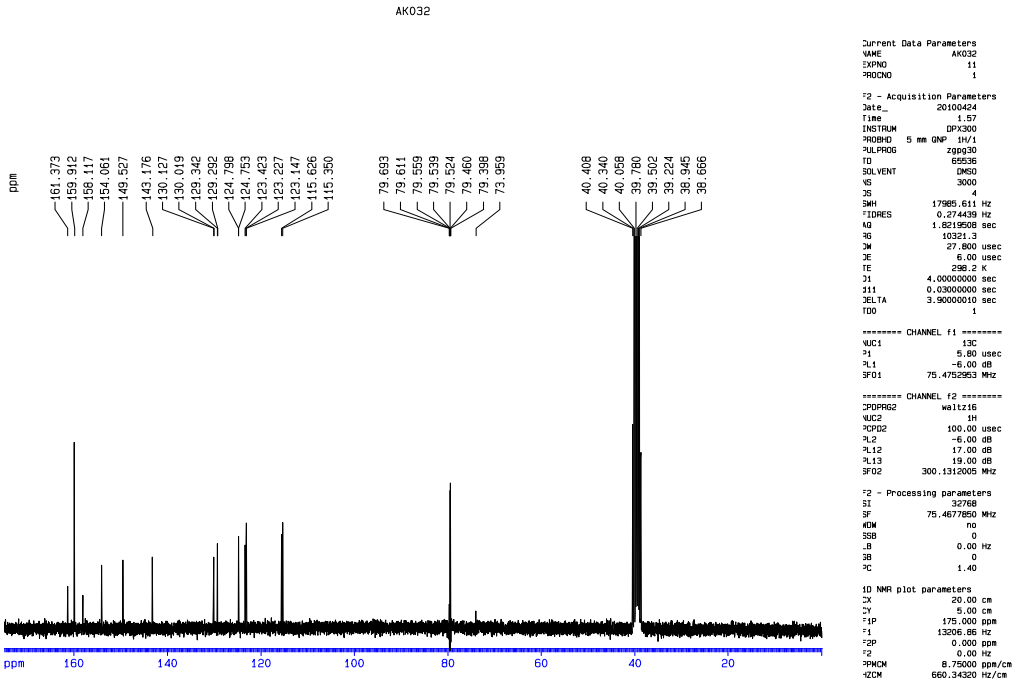
MS EI *m/z* (rel. %): 279/277 (24/70, M^+), 276 (6), 109 (100), 83 (11).

HR-MS Found 277.0532, calcd. for $C_{12}H_9ClFN_5$ 277.0531.

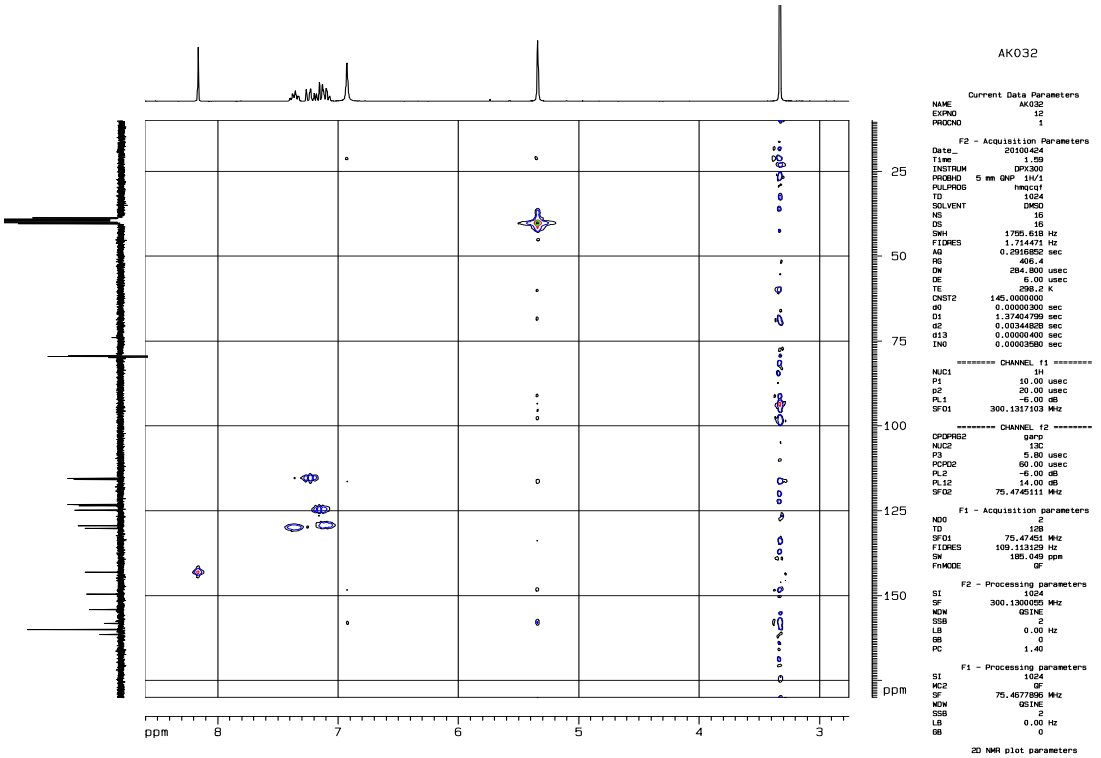
M.p. 190 ºC



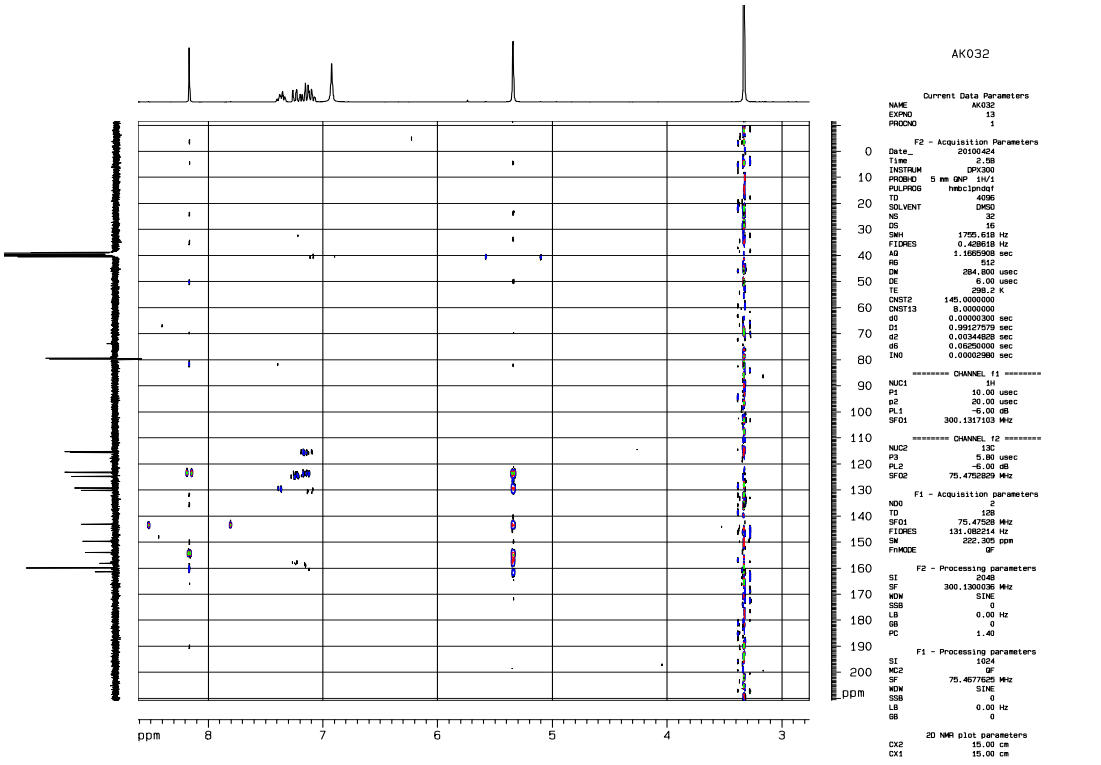
Spectrum 17. [1]H of 6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a).**



Spectrum 18. [13]C of 6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a).**



Spectrum 19. HMQC of 6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a).**



Spectrum 20. HMBC of 6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a).**

6-Chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b)**

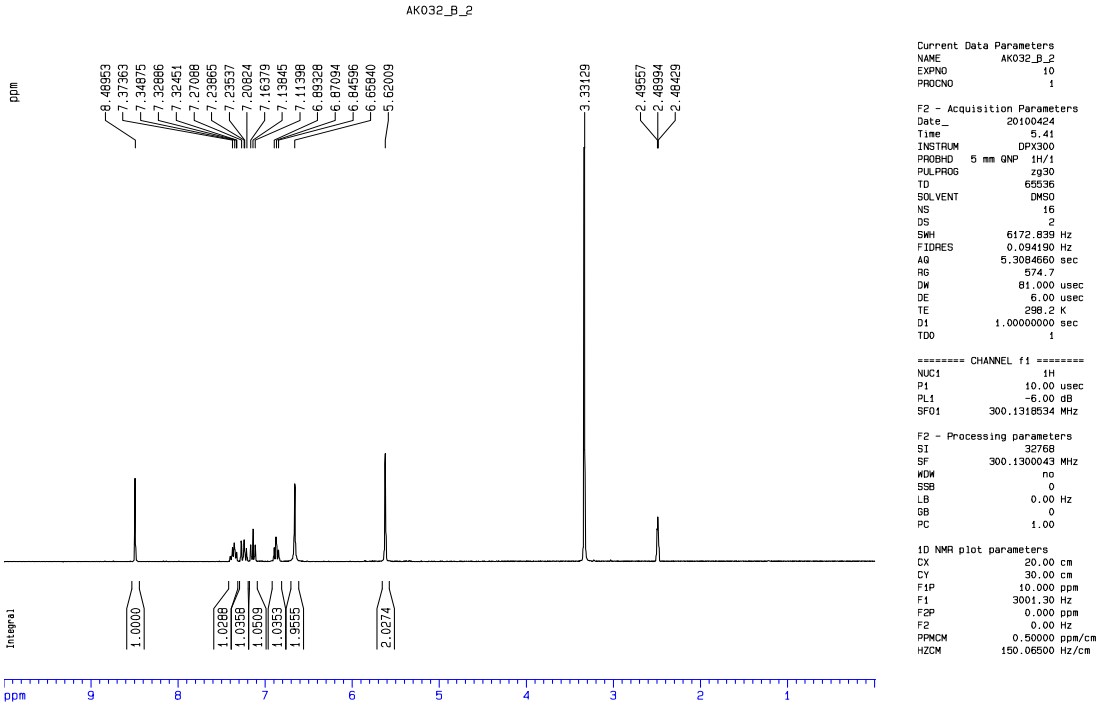
^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.50 (s, 1H, H-8), 7.41-7.34 (m, 1H, CH in Ar), 7.28-7.22 (m, 1H, CH in Ar), 7.18-7.12 (m, 1H, CH in Ar), 6.90-6.85 (m, 1H, CH in Ar), 6.67 (br s, 2H, NH_2), 5.63 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 165.2 (C-2), 160.9 (C-6), 160.2 (d, J_{CF} = 245 Hz, CF in Ar), 151.1 (C-8), 143.2 (C-4), 130.8 (d, J_{CF} = 8.2 Hz, CH in Ar), 129.0 (d, J_{CF} = 3.8 Hz, CH in Ar), 125.8 (d, J_{CF} = 3.5 Hz, CH in Ar), 125.1 (d, J_{CF} = 14.0 Hz, C in Ar), 116.3 (d, J_{CF} = 20.7 Hz, CH in Ar), 115.7 (C-5), 44.6 (CH_2).

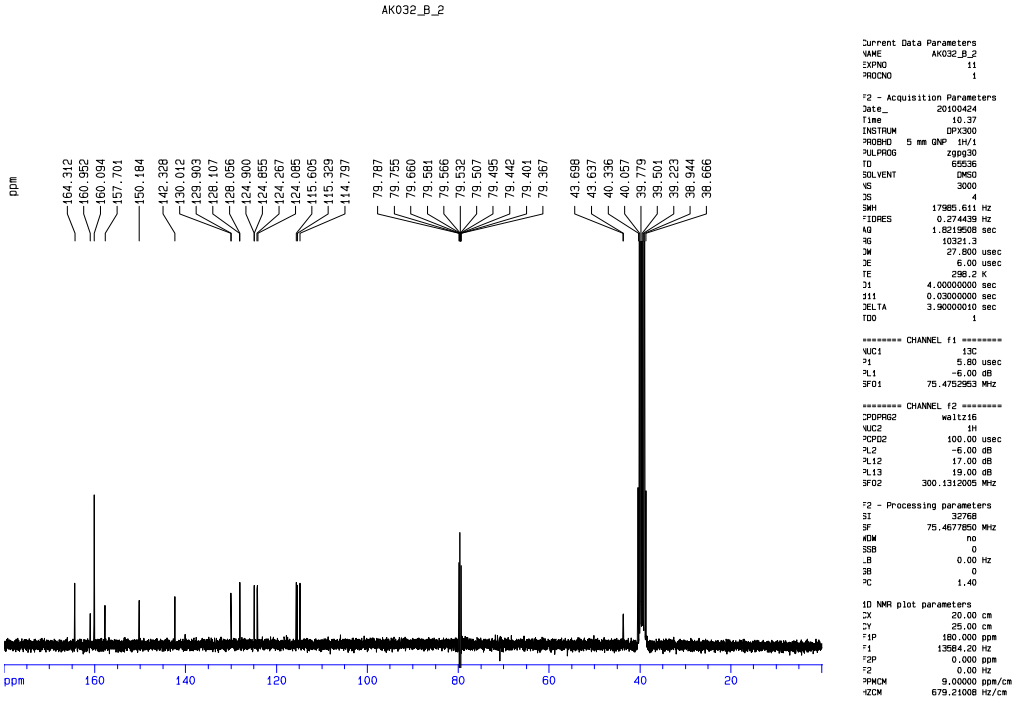
MS EI *m/z* (rel. %): 279/279 (18/56, M^+), 109 (100), 83 (10).

HR-MS Found 277.0532, calcd. for $C_{12}H_9ClFN_5$ 277.0531.

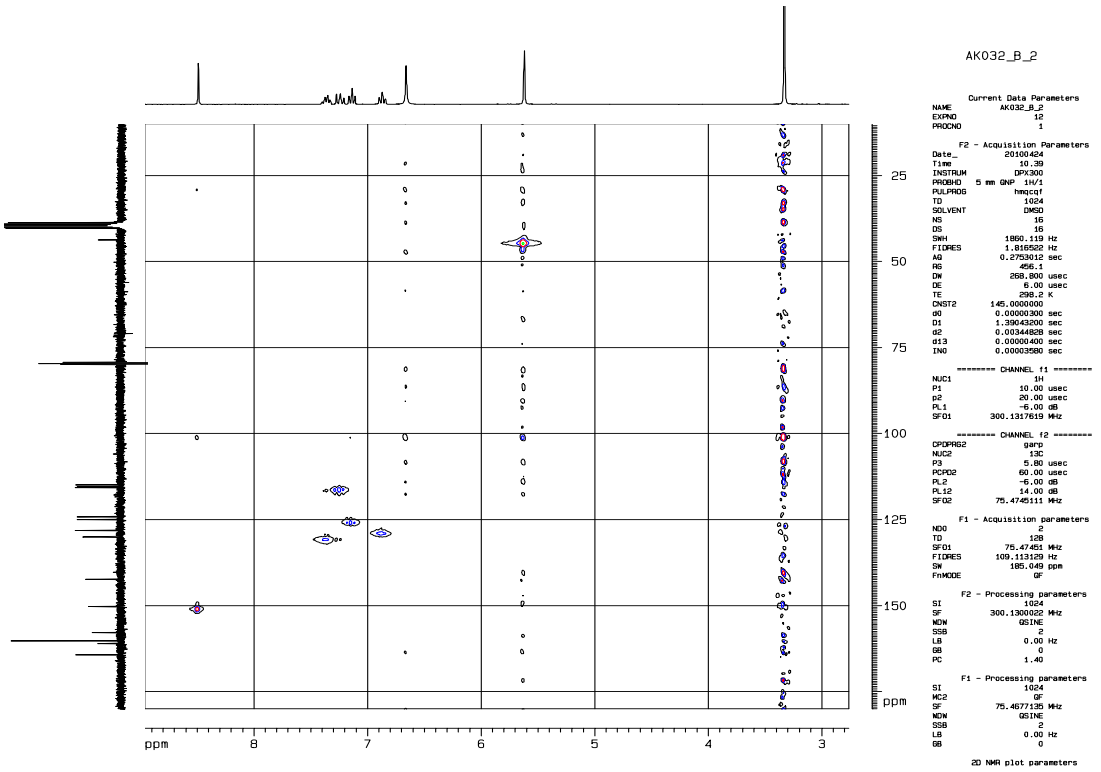
M.p. 266 ºC



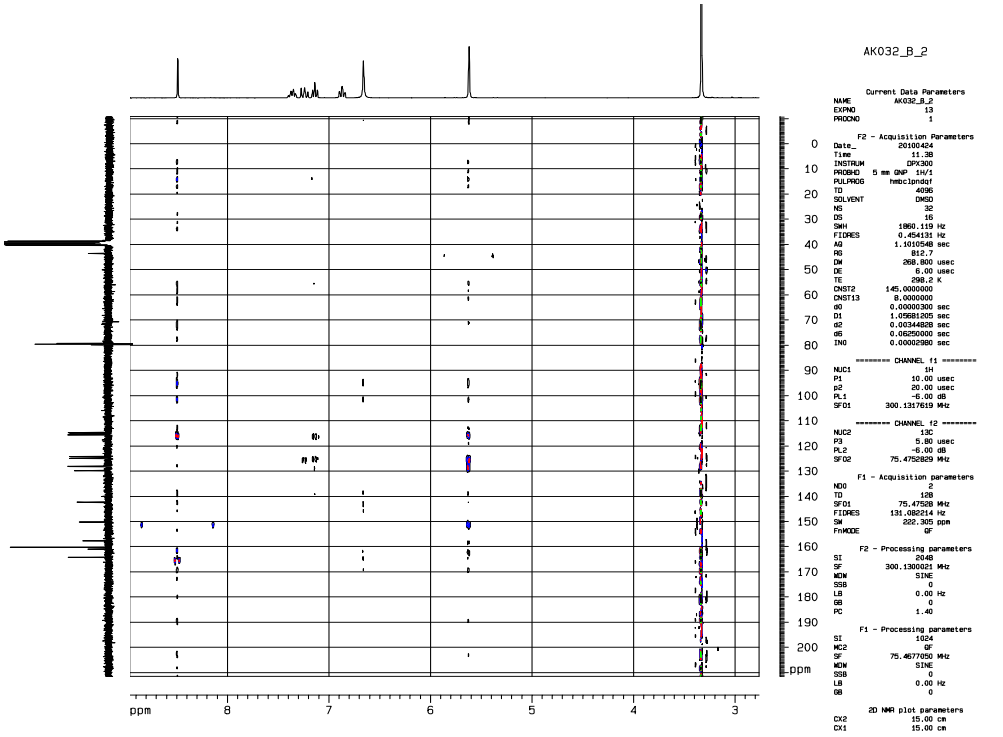
Spectrum 21. ^{1}H of 6-Chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b)**



Spectrum 22. ^{13}C of 6-Chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b).**

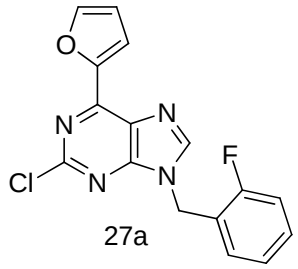


Spectrum 23. HMQC of 6-Chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b)**



Spectrum 24. HMBC of 6-Chloro-7-(2-fluorobenzyl)-7*H*-purin-2-amine **(26b).**

Synthesis of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine (27a)



27a

Method 1

A mixture of tris(dibenzylideneacetone)dipalladium chloroform adduct (16 mg, 0.02 mmol) and tri(2-furyl)phosphine (26 mg, 0.11 mmol) in DMF (4 mL) was stirred at r.t. under N_2 for 15 min., before 2,6-dichloro-9-(2-fluorobenzyl)-9*H*-purine **(23a/AN18a)** (150 mg, 0.50 mmol) and 2-(tributylstannyl)furan (0.20 mL, 0.64 mmol) were added. The resulting mixture was stirred for 18 h at 50 ºC, cooled and evaporated *in vacuo.* KF (sat. sol. in THF, 10 mL) was added and the resulting mixture was stirred at r.t. for 18 h. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (2:3) followed by pure ethyl acetate. This gave 82 mg (50%) of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine **(27a)** as a colourless powder.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.09 (s, 1H, H-8), 7.82 (dd, *J* = 3.5 Hz and 0.7 Hz, 1H, H-3 in furyl), 7.73 (dd, *J* = 1.7 Hz and 0.7 Hz, 1H, H-5 in furyl), 7.38 - 7.27 (m, 2H, H-3 and H-4 in Ar), 7.13 - 7.04 (m, 2H, H-5 and H-6 in Ar), 6.63 (dd, *J* = 3.5 Hz and 1.7 Hz, 1H, H-4 in furyl), 5.43 (s, 2H, CH_2).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 160.7 (d, J_{CF} = 247 Hz, CF in Ar), 154.5 (C-2), 153.5 (C-4), 148.7 (C-2 in furyl), 147.3 (C-6), 146.7 (C-4 in furyl), 144.9 (C-8), 130.9 (d, J_{CF} = 8.2 Hz, CH in Ar), 130.7 (d, J_{CF} = 3.2 Hz, CH in Ar), 126.9 (C-5) , 124.8 (d, J_{CF} = 3.6 Hz,

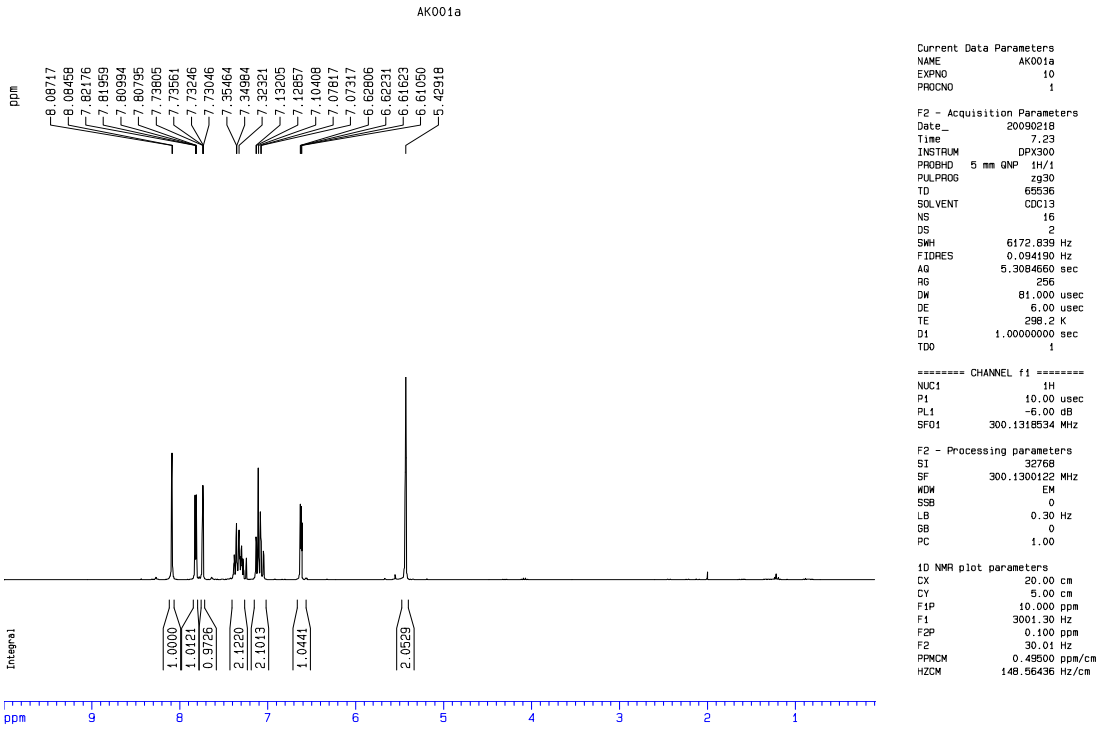
CH in Ar), 121.8 (d, J_{CF} = 14.5 Hz, C in Ar), 118.7 (C-3 in furyl), 115.8 (d, J_{CF} = 21.1 Hz, CH in Ar), 112.8 (C-5 in furyl), 41.3 (CH_2).

MS EI *m/z* (rel. %): 330/328 (17/52 M^+), 309 (8), 293 (4), 109 (100), 83 (15).

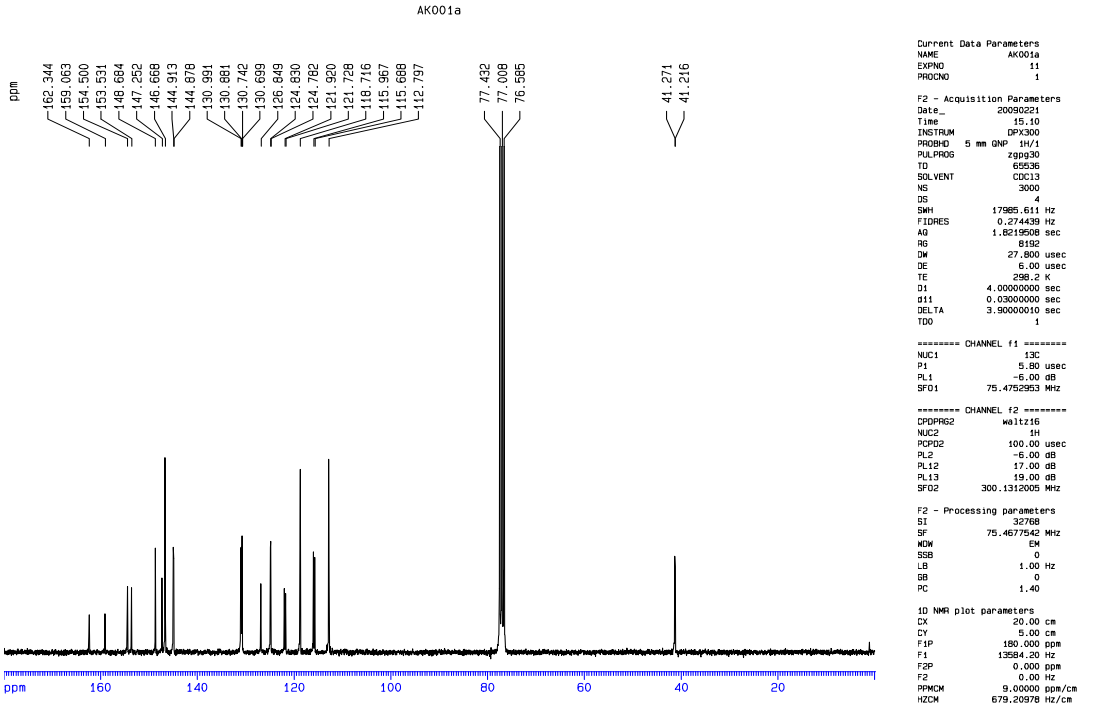
HR-MS Found 328.0532, calcd. for $C_{16}H_{10}ClFN_4O$ 328.0527.

Anal.: Found C, 58.53; H, 2.98; N, 16.59. $C_{16}H_{10}ClFN_4O$ requires C, 58.46; H, 3.07; N, 17.04.

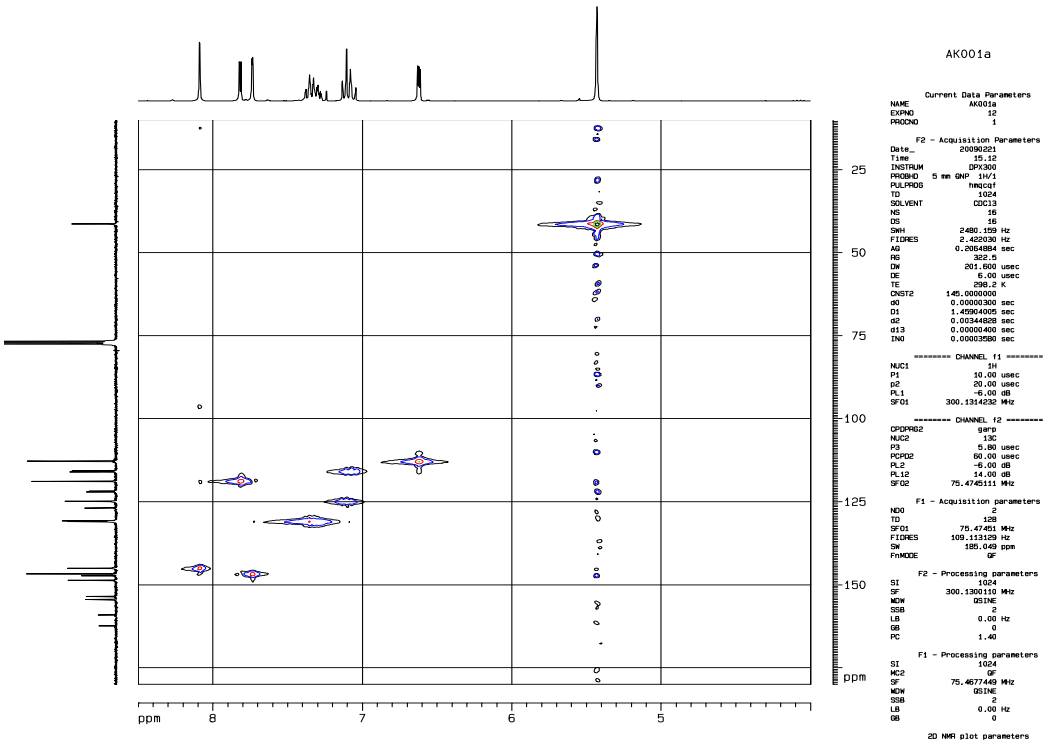
M.p. 138 ºC



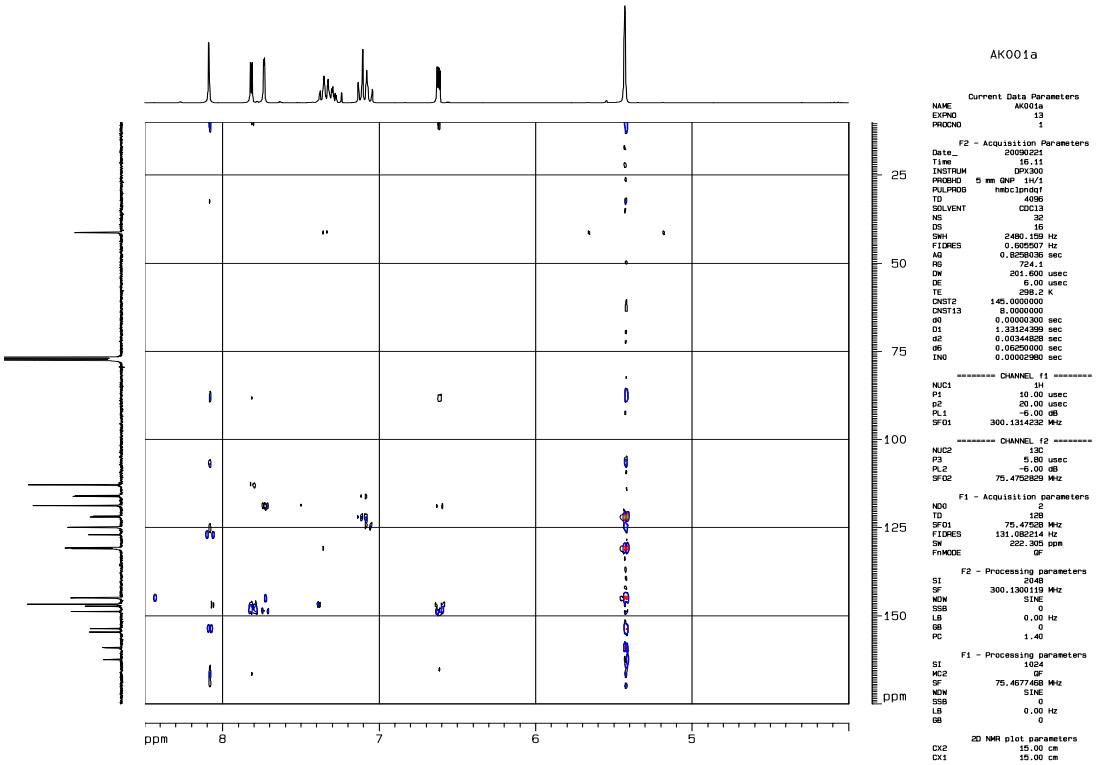
Spectrum 25. ^{1}H of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine **(27a)**



Spectrum 26. ^{13}C of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine **(27a).**

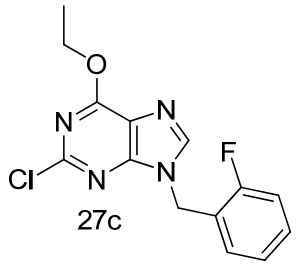


Spectrum 27. HMQC of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine **(27a)**



Spectrum 28. HMBC of 2-chloro-9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purine **(27a).**

2-Chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine (27c)



27c

2,6-Dichloro-7-(4-chlorobenzyl)-7*H*-purine **(23a)** (593 mg, 2.00 mmol), potassium furan-2-yltrifluoroborat (0.45 g, 2.56 mmol), K_2CO_3 (0.83 g, 6.0 mmol) and bis(triphenylphosphine)palladium (II) chloride (43 mg, 0.06 mmol) were added in ethanol (200 mL). The resulting mixture was stirred under N_2 for 2 h at 30 ºC, 30 min at 45 ºC and for 1.5 h at 50 ºC. The resulting crude mixture was purified by dry flash chromatography on silica gel using ethanol and filtrated with a mixture of ethanol/cold water. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (2:4) followed by pure ethyl acetate. This gave 220 mg (36%) of 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c)** as a colourless powder.

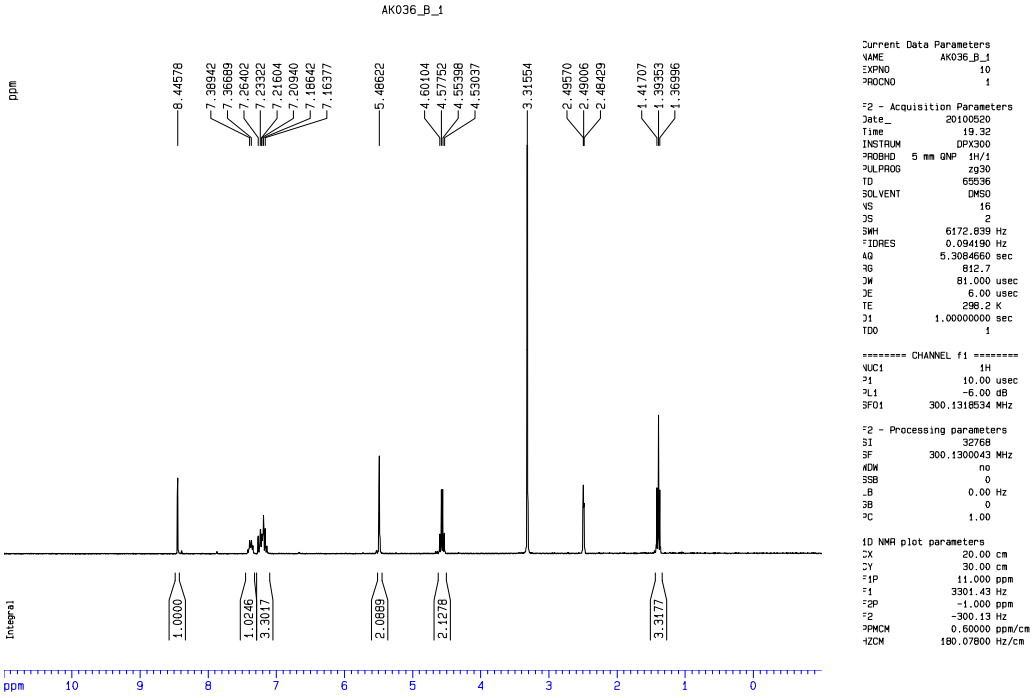
^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.46 (s, 1H, H-8), 7.45-7.13 (m, 4H in Ar), 5.49 (s, 2H, CH_2), 4.57 (q, J = 7.07 Hz, 2H, CH_2), 1.40 (t, J = 7.08 Hz, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.4 (C-6 or C-2), 159.8 (d, J_{CF} = 246 Hz, CF in Ar), 153.1 (C-4), 151.4 (C-2 or C-6), 144.4 (C-8), 130.3 (d, J_{CF} = 8.2 Hz, CH in Ar), 129.8 (d, J_{CF} = 3.7 Hz, CH in Ar), 124.7 (d, J_{CF} = 3.6 Hz, CH in Ar), 122.8 (d, J_{CF} = 14.5 Hz, C in Ar), 119.6 (C-5), 115.5 (d, J_{CF} = 20.7 Hz, CH in Ar), 63.7 (OCH_2), 40.9 (CH_2), 14.1 (CH_3).

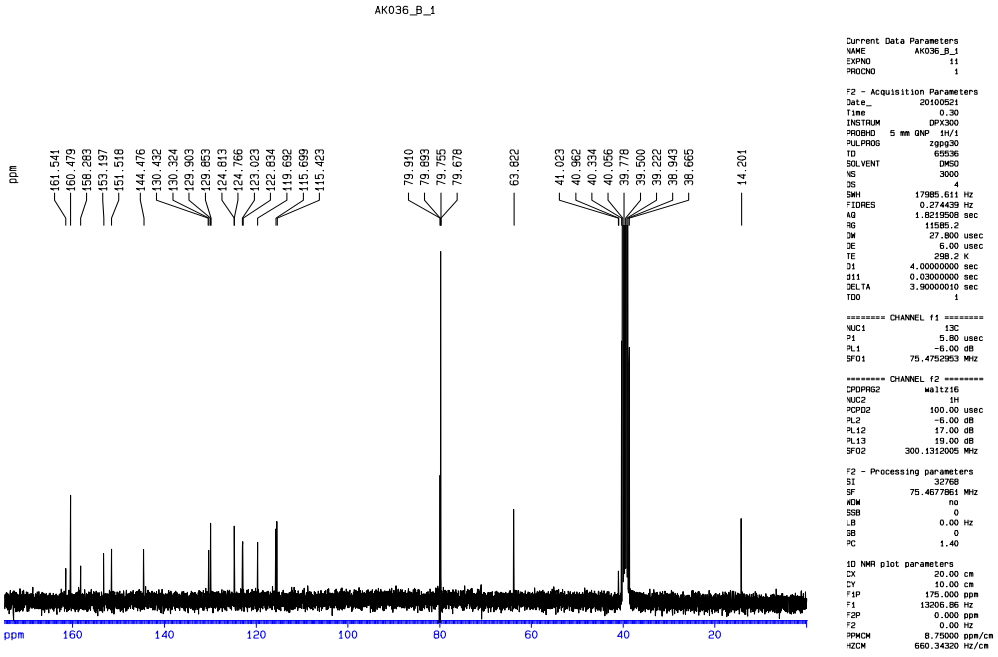
MS EI *m/z* (rel. %): 308/306 (6/18, M^+), 293/291 (5/15), 278/280 (11/4), 262/264 (6/2), 227 (10), 109 (100), 83 (12).

HR-MS Found 306.0682, calcd. for $C_{14}H_{12}ClFN_4O$ 306.0684.

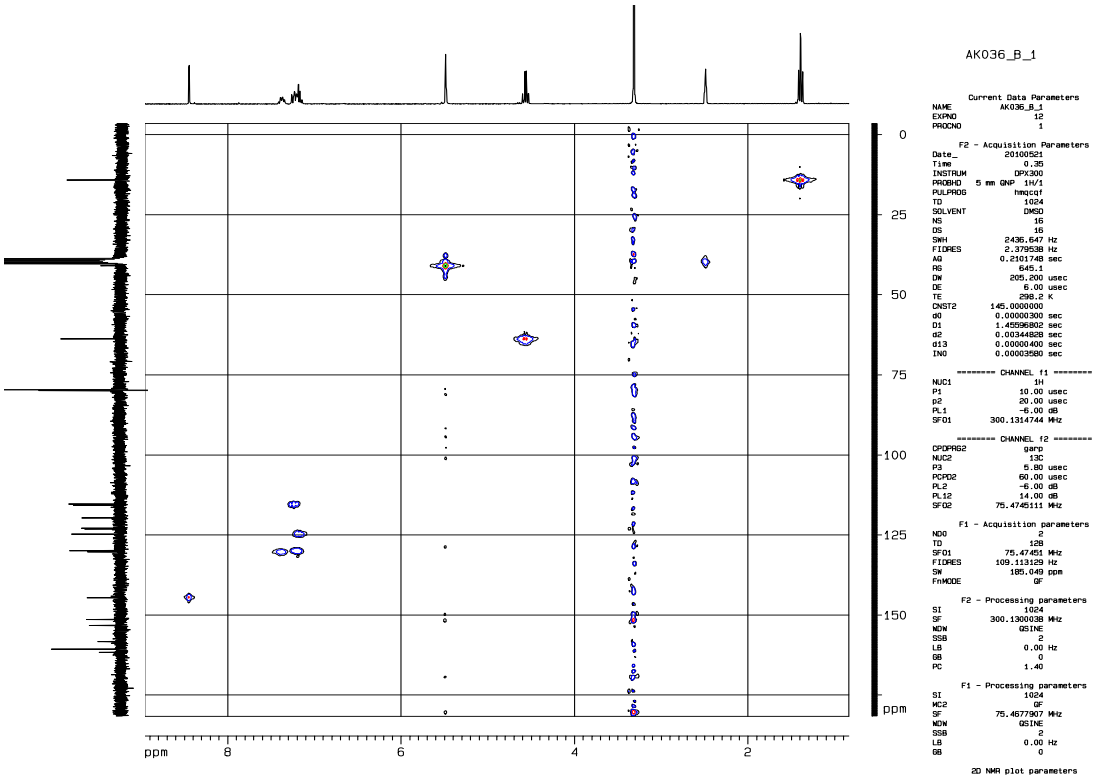
M.p. 110 ºC



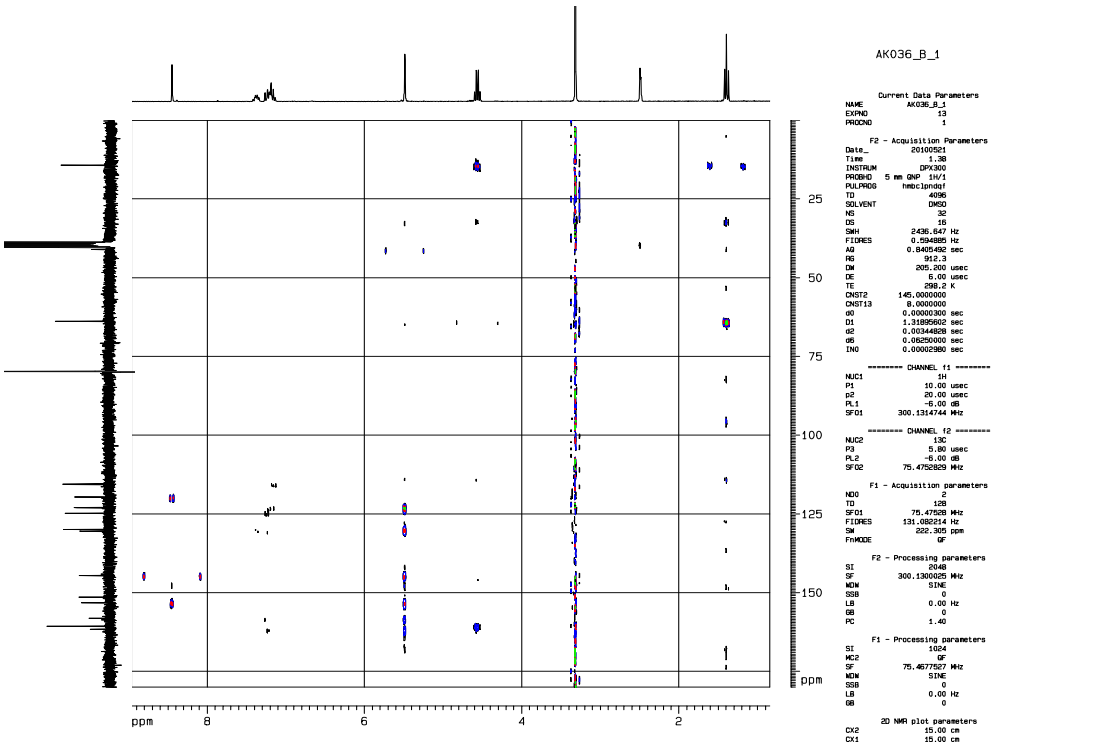
Spectrum 29. ^{1}H of 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c)**



Spectrum 30. ^{13}C of 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c).**

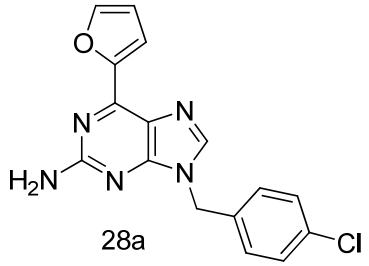


Spectrum 31. HMQC of 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c)**



Spectrum 32. HMBC of 2-chloro-6-ethoxy-9-(2-fluorobenzyl)-9*H*-purine **(27c).**

Synthesis of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine (28a)



28a

Method 1

A mixture of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** (155 mg, 0.53 mmol), bis(triphenylphosphine)palladium (II) chloride (18 mg, 0.03 mmol) and 2-(tributylstannyl)furan (0.25 mL, 0.80 mmol) in DMF (2.6 mL) was stirred at 90 ºC under N_2 for 18 h, cooled and evaporated *in vacuo.* KF (sat. sol. in MeOH, 2.6 mL) was added and the resulting mixture was stirred at r.t. for 18 h. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (1:1). This gave 104 mg (61 %) of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** as a yellow powder.

Method 2

A mixture of tris(dibenzylideneacetone)dipalladium chloroform adduct (38.5 mg, 0.04 mmol) and tri(2-furyl)phosphine (63.3 mg, 0.27 mmol) in DMF (9.8 mL) was stirred at r.t. under N_2 for 15 min., before 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** (365 mg, 1.24 mmol) and 2-(tributylstannyl)furan (0.50 mL, 0.59 mmol) were added. The resulting mixture was stirred for 18 h at 90 ºC, cooled and evaporated *in vacuo.* KF (sat.

sol. in THF, 9.8 mL) was added and the resulting mixture was stirred at r.t. for 18 h. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (1:1) followed by pure ethyl acetate. This gave 110 mg (27 %) of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** as a yellow powder.

Method 3

A mixture of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** (588 mg, 2.00 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium (II) (82 mg, 0.1 mmol), 2-(tributylstannyl)furan (0.81 mL, 2.56 mmol), LiCl (0.25 g, 6.0 mmol) and CsF (0.90 g, 6.0 mmol) in DMF (25 mL) was stirred at 90 ºC under N_2 18 h, cooled and evaporated *in vacuo.* The resulting crude mixture was dry flashed on silica gel using acetone. KF (sat. sol. in THF, 25 mL) was added and the resulting mixture was stirred at r.t. for 18 h. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using DCM/methanol (98:2) followed by DCM/methanol (96:4). This gave 200 mg (31 %) of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** as a yellow powder.

Method 4

6-Chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(25a)** (588 mg, 2.00 mmol), potassium furan-2-yltrifluoroborat (0.45 g, 2.56 mmol), K_2CO_3 (0.83 g, 6.0 mmol), palladium (II) acetat (14 mg, 0.06 mmol) and triphenylphosphine (115 mg, 0.44 mmol) were added in ethanol (200 mL). The resulting mixture was stirred under N_2 for 1.5 h at 70 ºC and 1.5 h at 80 ºC. The resulting crude mixture was purified by dry flash chromatography on silica gel using ethanol and filtrated with a mixture of hexane/ethanol/cold water. The mixture was evaporated *in vacuo.* Ethyl acetate and hexane were added and the product precipitated. This gave 375 mg (50 %) of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)** as a yellow powder.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.20 (s, 1H, H-8), 7.79 (dd, J = 1.7 Hz and 0.7 Hz, 1H, H-5 in furyl), 7.72 (dd, J = 3.4 Hz and 0.7 Hz, 1H, H-3 in furyl), 7.40 (d, J = 8.6 Hz, 2H in Ar), 7.28 (d, J = 8.6 Hz, 2H in Ar), 6.74 (dd, J = 3.4 Hz and 1.7 Hz, 1H, H-4 in furyl) , 6.59 (br s, 2H, NH_2), 5.32 (s, 2H, CH_2).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.4 (C-2), 153.7 (C-4), 149.4 (C-2 in furyl), 145.6 (C-6), 145.4 (C-5 in furyl), 142.4 (C-8), 136.0 (C in Ar), 132.3 (C-4 in Ar), 128.7 (CH in Ar), 128.1 (CH in Ar), 121.6 (C-5), 116.4 (C-3 in furyl), 112.4 (C-4 in furyl), 45.0 (CH_2).

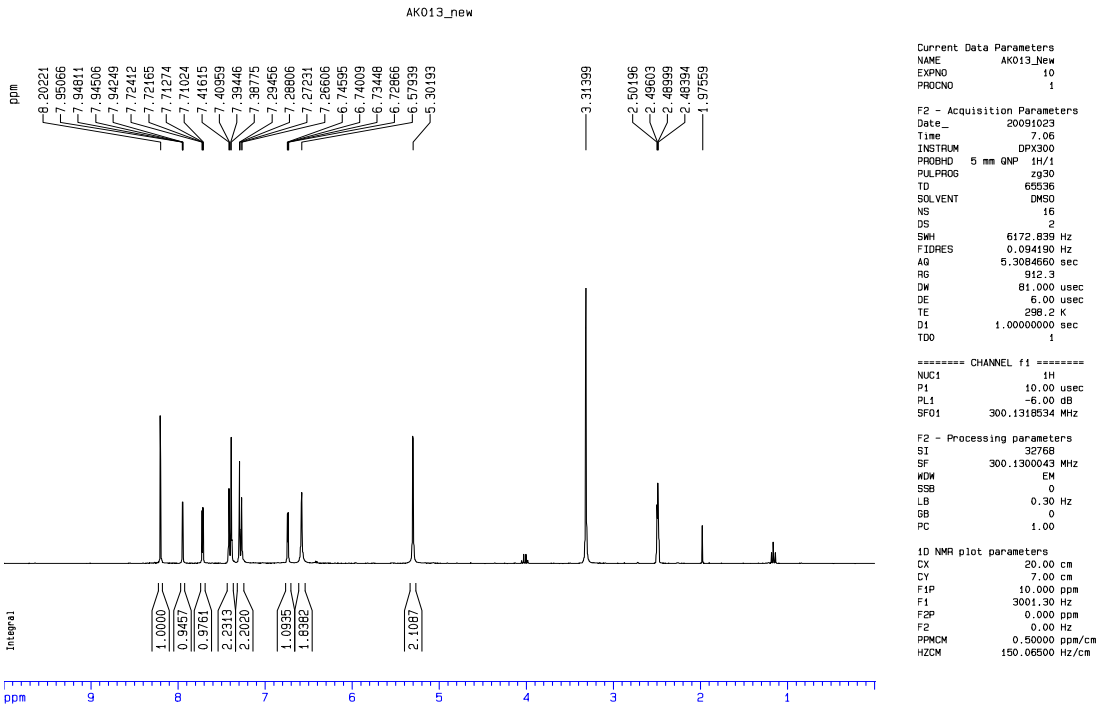
MS EI *m/z* (rel. %): 327/325 (33/100, M^+), 324 (23), 214 (5), 200 (22), 127/125 (19/61), 89 (8).

HR-MS Found 325.0731, calcd. for $C_{16}H_{12}ClN_5O$ 325.0730.

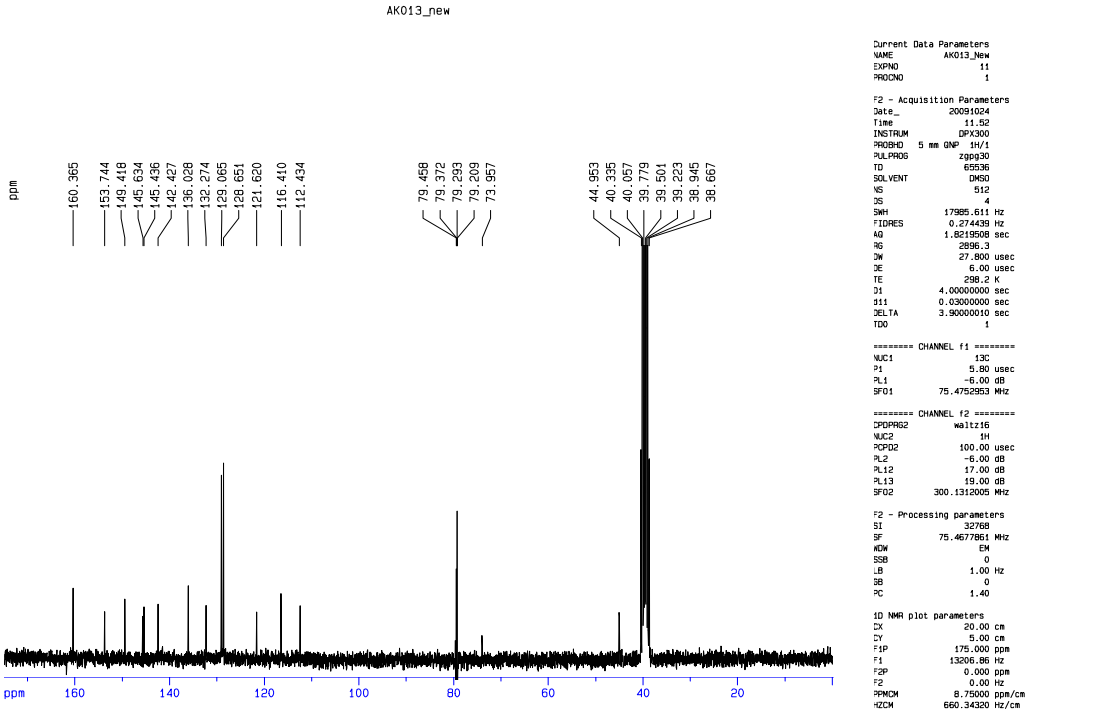
Anal.: Found C, 58.67; H, 3.59; N, 21.33. $C_{16}H_{12}ClN_5O$ requires C, 58.99; H, 3.71; N, 21.50.

M.p. 178 ºC

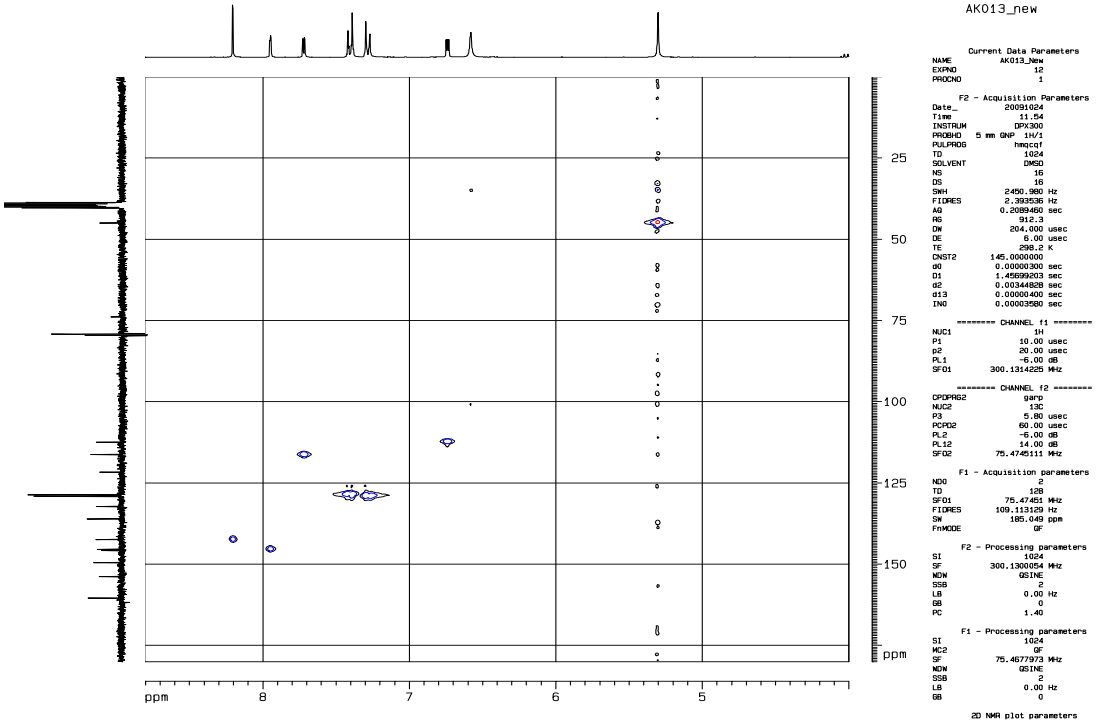
Compound **28a** is known in literature.[20]



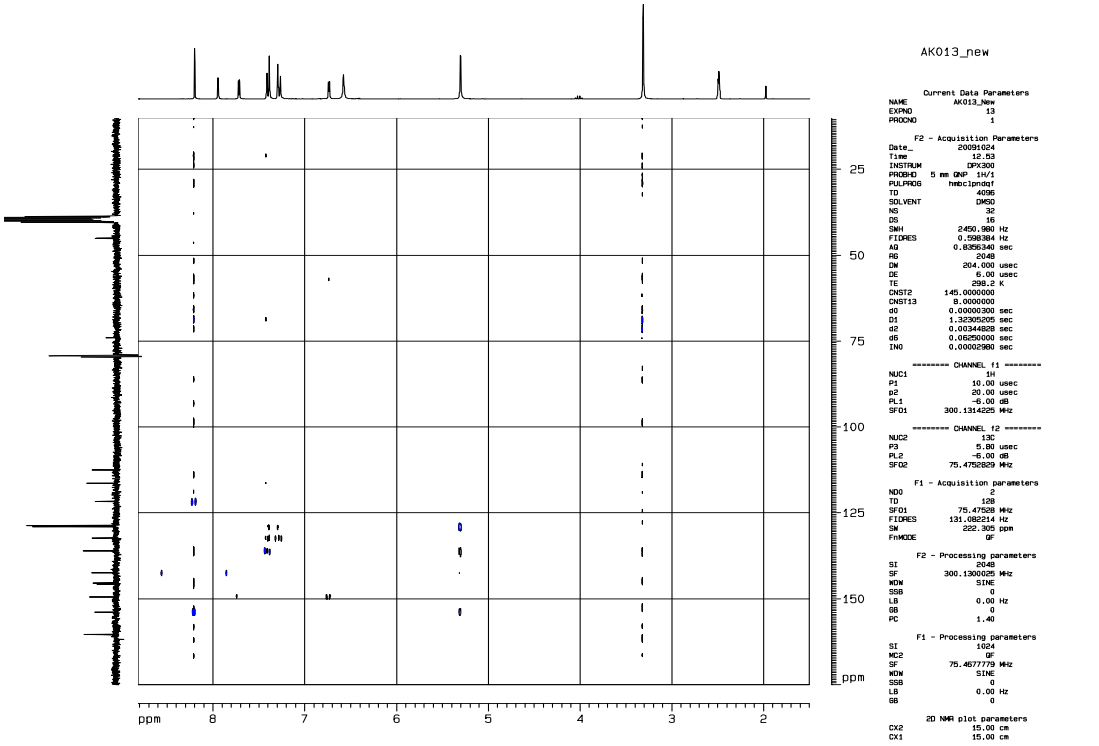
Spectrum 33. ^{1}H of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)**



Spectrum 34. ^{13}C of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a).**

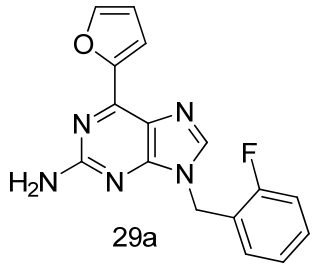


Spectrum 35. HMQC of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a)**



Spectrum 36. HMBC of 9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(28a).**

Synthesis of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine (29a)



29a

Method 1

A mixture of 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** (554 mg, 2.00 mmol), [1,1'-Bis(diphenylphosphino)-ferrocene]dichloropalladium (II) (82 mg, 0.1 mmol), 2-(tributylstannyl)furan (0.81 mL, 2.56 mmol), LiCl (0.25 g, 6.0 mmol) and CsF (0.90 g, 6.0 mmol) in DMF (25 mL) was stirred at 90 ºC under N_2 for 18 h, cooled and evaporated *in vacuo.* The resulting crude mixture was purified by dry flash chromatography on silica gel using acetone. KF (sat. sol. in THF, 25 mL) was added and the resulting mixture was stirred at r.t. for 18 h. The mixture was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using DCM/methanol (98:2). This gave 180 mg (29 %) of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** as a colourless powder.

Method 2

6-Chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** (554 mg, 2.00 mmol), potassium furan-2-yltrifluoroborat (0.45 g, 2.56 mmol), K_2CO_3 (0.83 g, 6.0 mmol), palladium (II) acetat (14 mg, 0.06 mmol) and triphenylphosphine (115 mg, 0.44 mmol) were added in ethanol (67 mL). The resulting mixture was stirred under N_2 for 3.5 h at 75 ºC and 30 min. at 85 ºC. Ethanol and water were added to the crude mixture and heated up to the boiling point. The solution was cooled in fridge over night. The mixture was filtrated with hot hexane. The remaining product in the filter paper was then filtrated with hot methanol. The product was evaporated *in vacuo.* This gave 510 mg (83 %) of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** as a colourless powder.

Method 3

A mixture of 6-chloro-9-(2-fluorobenzyl)-9*H*-purin-2-amine **(26a)** (554 mg, 2.00 mmol), potassium furan-2-yltrifluoroborat (0.45 g, 2.56 mmol), K_2CO_3 (0.83 g, 6.0 mmol), palladium (II) acetat (14 mg, 0.06 mmol) and triphenylphosphine (115 mg, 0.44 mmol) in ethanol (200 mL) was stirred under N_2 for 4 h at 80 ºC. The mixture was filtered and evaporated *in vacuo*. The product was purified by flash chromatography on silica gel using DCM/methanol (97:3) and crystallized from DCM/chloroform. This gave 445 mg (72 %) of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** as a colourless powder.

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.16 (s, 1H, H-8), 7.96 (dd, J = 1.8 Hz and 0.8 Hz, 1H, H-5 in furyl), 7.73 (dd, J = 3.4 Hz and 0.8 Hz, 1H, H-3 in furyl), 7.42-7.04 (m, 4H, CH in Ar), 6.74 (dd, J = 3.4 Hz and 1.8 Hz, 1H, H-4 in furyl), 6.59 (br s, 2H, NH_2), 5.37 (s, 2H, CH_2).

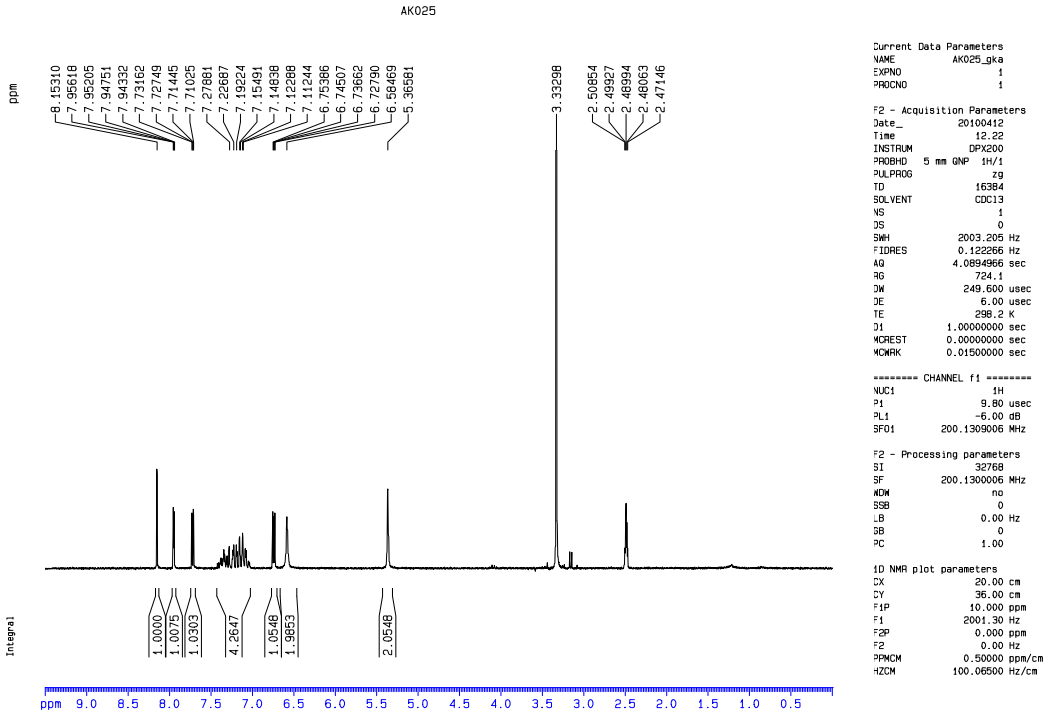
^{13}C NMR (DMSO-d_6, 75 MHz): δ 160.3 (C-2), 159.7 (d, J_{CF} = 245 Hz, CF in Ar), 153.7 (C-4), 149.3 (C-2 in furyl), 145.6 (C-6), 145.4 (C-5 in furyl), 142.4 (C-8), 129.9 (d, J_{CF} = 8.2 Hz, CH in Ar), 129.2 (d, J_{CF} = 3.8 Hz, CH in Ar), 124.7 (d, J_{CF} = 3.5 Hz, CH in Ar), 123.6 (d, J_{CF} = 14.6 Hz, C in Ar), 121.4 (C-5), 116.3 (C-3 in furyl), 115.4 (d, J_{CF} = 20.7 Hz, CH in Ar), 112.4 (C-4 in furyl), 39.7 (CH_2).

MS EI *m/z* (rel. %): 309 (100, M^+), 290 (6), 200 (28), 109 (49), 83 (6).

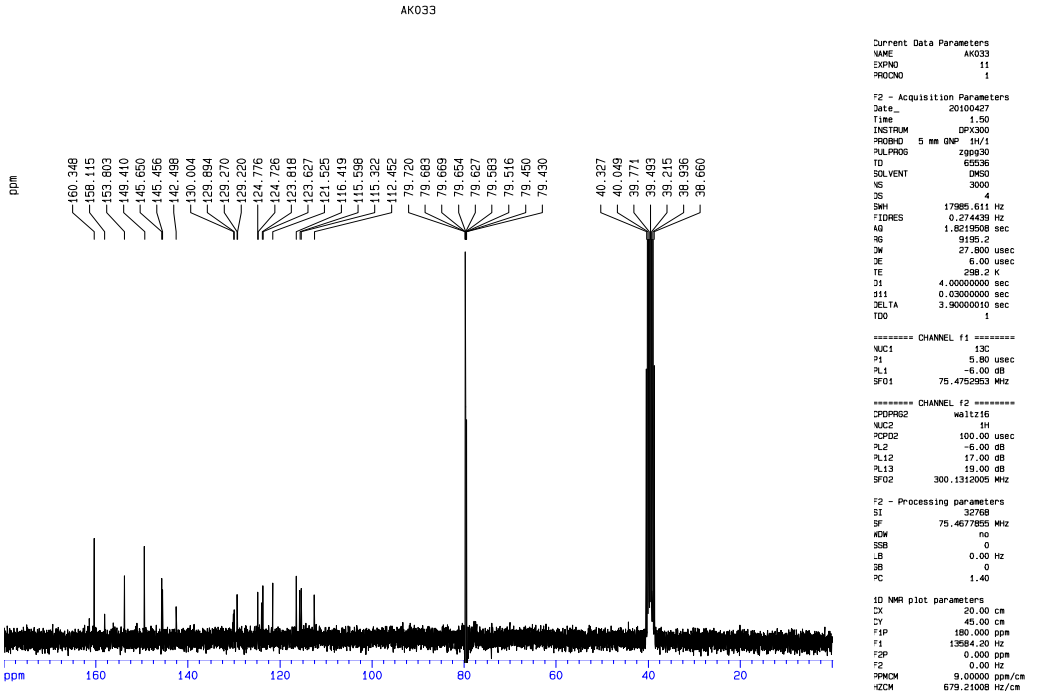
HR-MS Found 309.1029, calcd. for $C_{16}H_{12}FN_5O$ 309.1026.

Anal.: Found C, 62.44; H, 4.08; N, 22.62. $C_{16}H_{12}FN_5O$ requires C, 62.13; H, 3.91; N, 22.64.

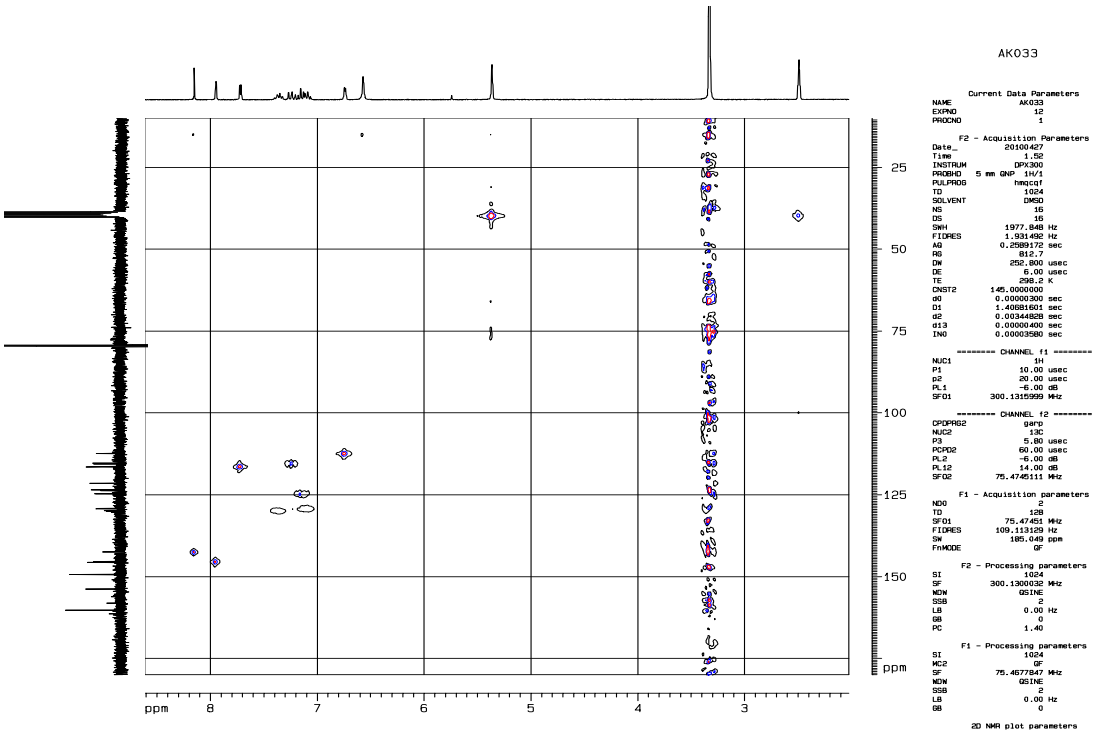
M.p. 192 ºC



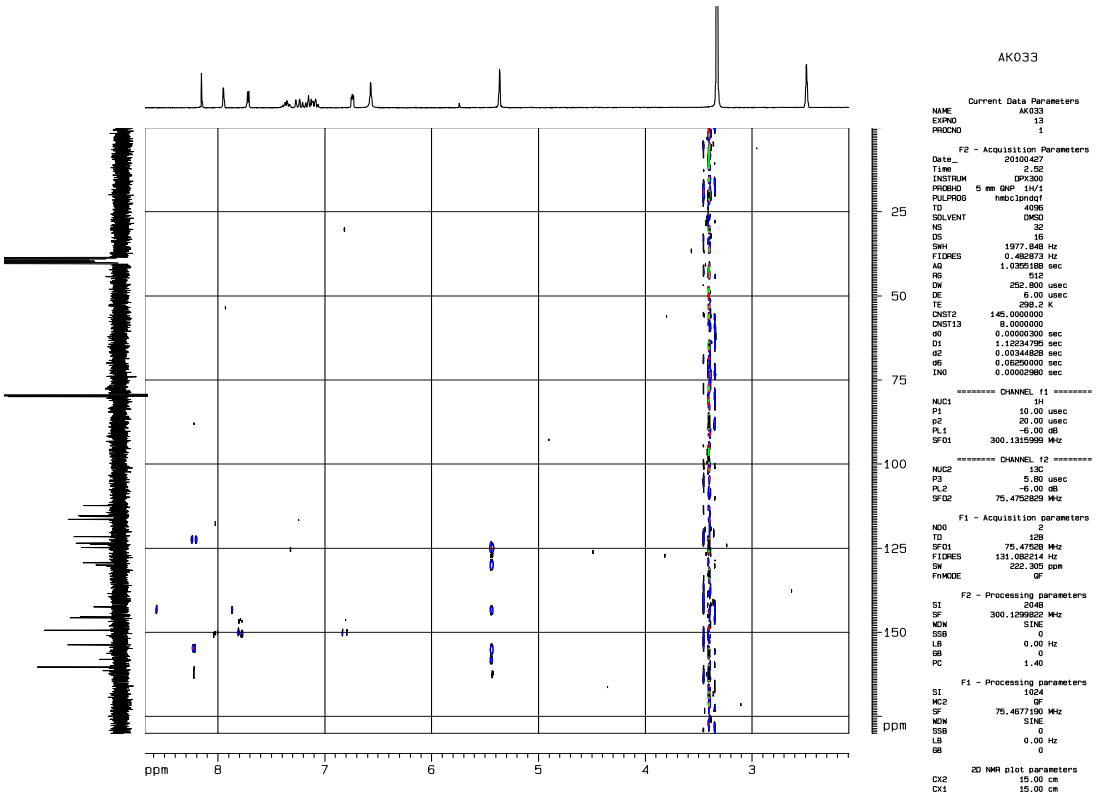
Spectrum 37. ^{1}H of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)**



Spectrum 38. ^{13}C of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a).**

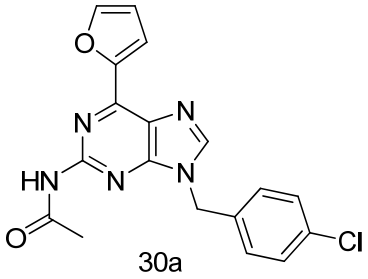


Spectrum 39. HMQC of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)**

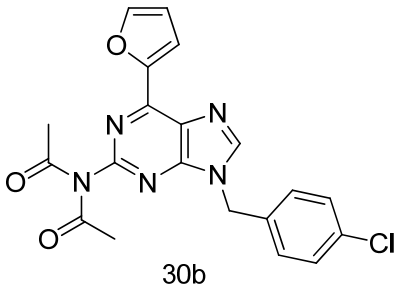


Spectrum 40. HMBC of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a).**

Synthesis of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide (30a) and *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide (30b)



30a



30b

Method 1

Acetic anhydride (0.27 mL, 2.90 mmol) was added to a stirred suspension of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(28a)** (101 mg, 0.33 mmol) and triethylamine (0.23 mL, 1.70 mmol) in toluene (3.30 mL) and the resulting mixture was heated at reflux for 24 h, cooled and evaporated *in vacuo.* The products were purified by flash chromatography on silica gel using ethyl acetate/hexane (2:1). This gave 64 mg (53 %) of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a)** as a pale yellow powder and 12 mg (9 %) of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)** as a colourless powder.

Method 2

A stirred suspension of 6-chloro-9-(4-chlorobenzyl)-9*H*-purin-2-amine **(28a)** (110 mg, 0.36 mmol) in toluene (3 mL) was heated at reflux for 24 h. Acetic anhydride (0.34 mL, 3.60 mmol) was added. After additional 24 h reflux, the solution was evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl

acetate/hexane (2:1). This gave 98 mg (66 %) of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)** as a colourless powder.

N-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a)**

^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.58 (br s, 1H, NH), 8.60 (s, 1H, H-8), 8.06 (dd, *J* = 1.7 Hz and 0.7 Hz, 1H, H-5 in furyl), 7.82 (dd, *J* = 3.5 Hz and 0.7 Hz, 1H, H-3 in furyl), 7.49 (d, *J* = 9.0 Hz, 2H in Ar), 7.41 (d, *J* = 9.0 Hz, 2H in Ar), 6.81 (dd, *J* = 3.5 Hz and 1.7 Hz, 1H, H-4 in furyl), 5.43 (s, 2H, CH_2), 2.24 (s, 3H, CH_3).

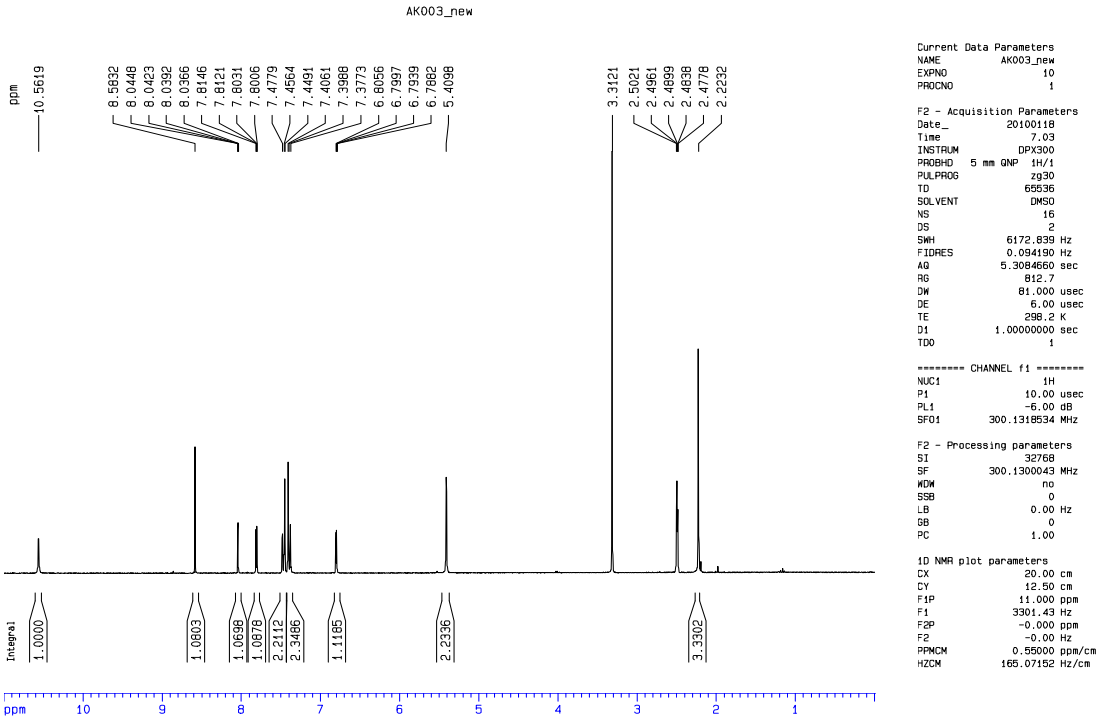
^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.0 (CO), 152.7 (C-4), 152.5 (C-2), 148.7 (C-2 in furyl), 146.3 (C-5 in furyl), 145.4 (C-8), 145.1 (C-6), 135.4 (C in Ar), 132.6 (C-4 in Ar), 129.8 (CH in Ar), 128.6 (CH in Ar), 124.5 (C-5), 117.5 (C-3 in furyl), 112.8 (C-4 in furyl), 45.6 (CH_2), 24.5 (CH_3).

MS EI *m/z* (rel. %): 369/367 (25/71, M^+), 327/325 (34/100), 214 (6), 200 (21), 127/125 (27/84), 89 (10).

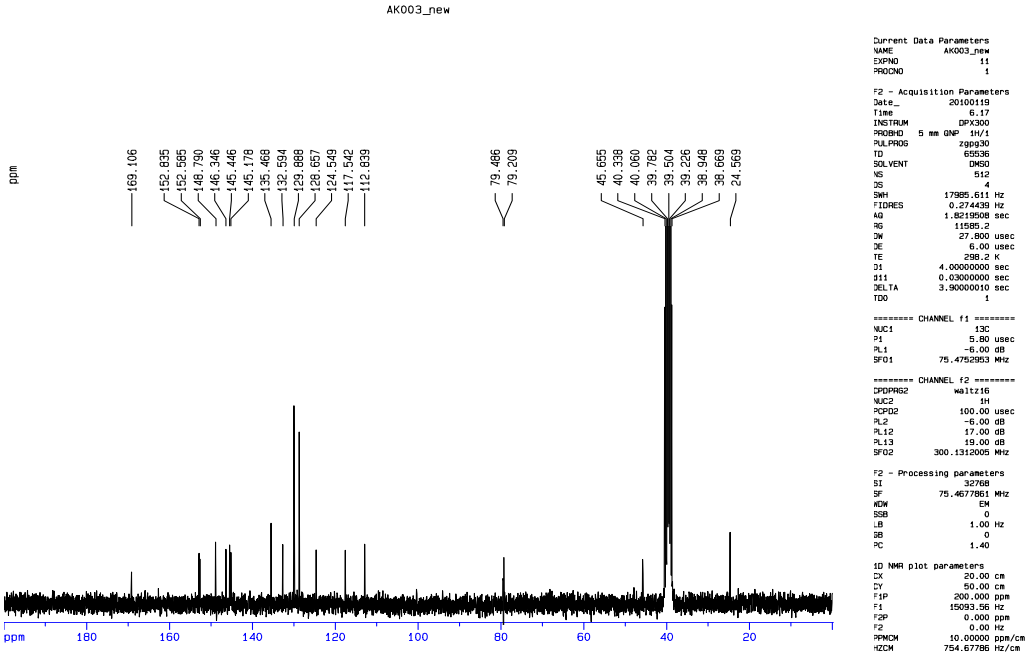
HR-MS Found 367.0829, calcd. for $C_{18}H_{14}ClN_5O_2$ 367.0836.

Anal.: Found C, 58.83; H, 3.86; N, 19.36. $C_{18}H_{14}ClN_5O_2$ requires C, 58.78; H, 3.84; N, 19.04.

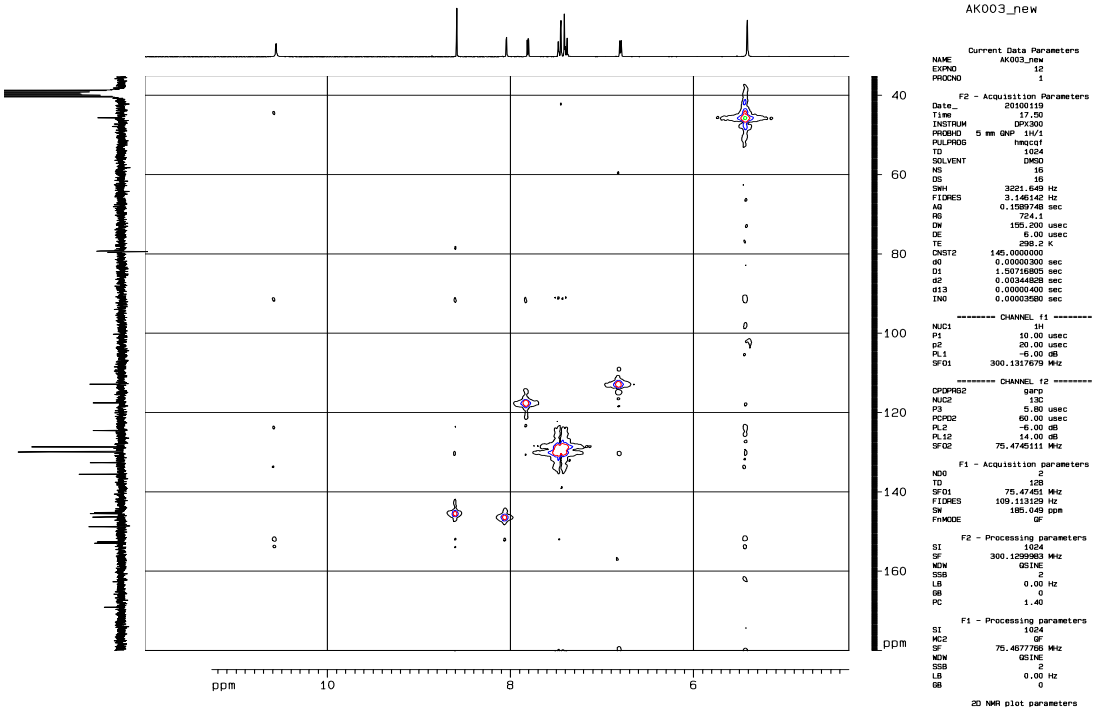
M.p. 190 ºC



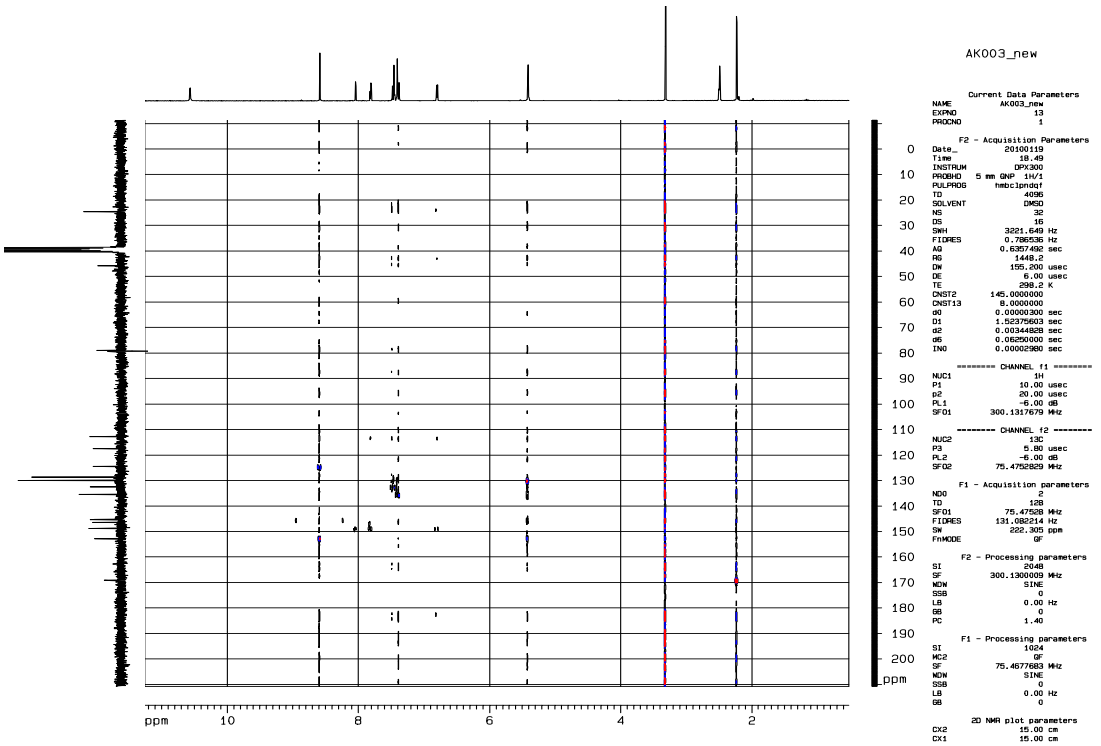
Spectrum 41. ^{1}H of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a).**



Spectrum 42. ^{13}C of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a).**



Spectrum 43. HMQC of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a).**



Spectrum 44. HMBC of *N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*purin-2-yl)acetamide **(30a).**

N-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)**

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.88 (s, 1H, C-8), 8.10 (dd, J = 1.7 Hz and 0.7 Hz, 1H, H-5 in furyl), 7.93 (dd, J = 3.5 Hz and 0.7 Hz, 1H, H-3 in furyl), 7.42 (d, J = 9.0 Hz, 2H in Ar), 7.38 (d, J = 9.0 Hz, 2H in Ar), 6.85 (dd, J = 3.5 Hz and 1.7 Hz, 1H, H-4 in furyl), 5.53 (s, 2H, CH_2), 2.20 (s, 6H, 2 × CH_3).

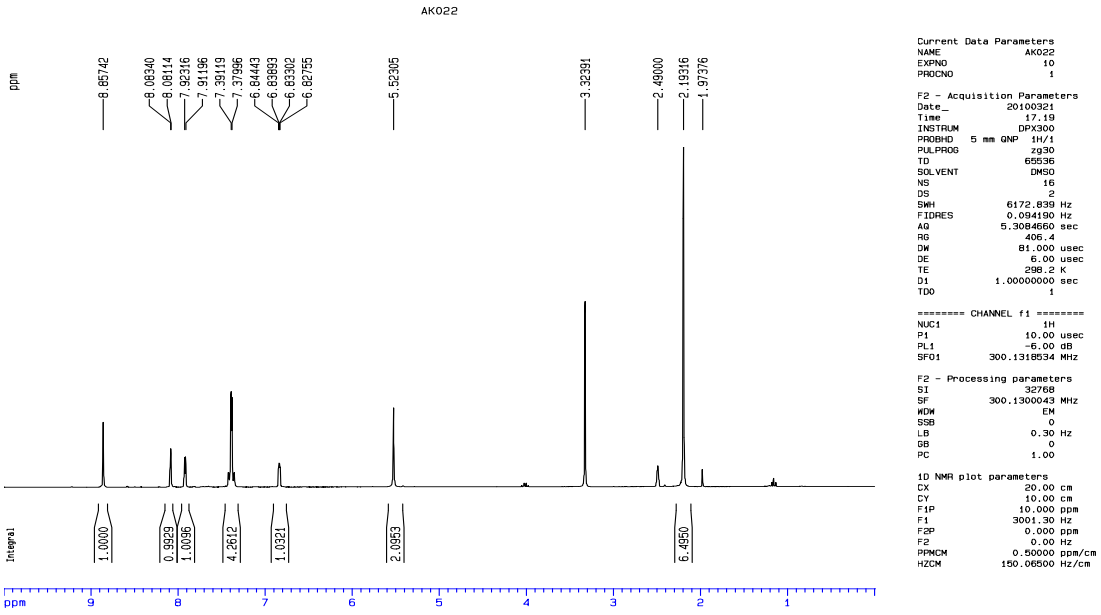
^{13}C NMR (DMSO-d_6, 75 MHz): δ 171.9 (2 × CO), 153.1 (C-4), 152.4 (C-2), 148.0 (C-8), 147.9 (C-2 in furyl), 147.0 (C-5 in furyl), 145.9 (C-6), 135.0 (C in Ar), 132.6 (C-4 in Ar), 129.5 (CH in Ar), 128.6 (CH in Ar), 126.9 (C-5), 118.6 (C-3 in furyl), 113.1 (C-4 in furyl), 45.9 (CH_2), 25.7 (2 × CH_3).

MS EI *m/z* (rel. %): 411/409 (4/14, M^+), 369/367 (16/48), 354/352 (27/83), 327/325 (17/53), 200 (11), 127/125 (30/100), 89 (10).

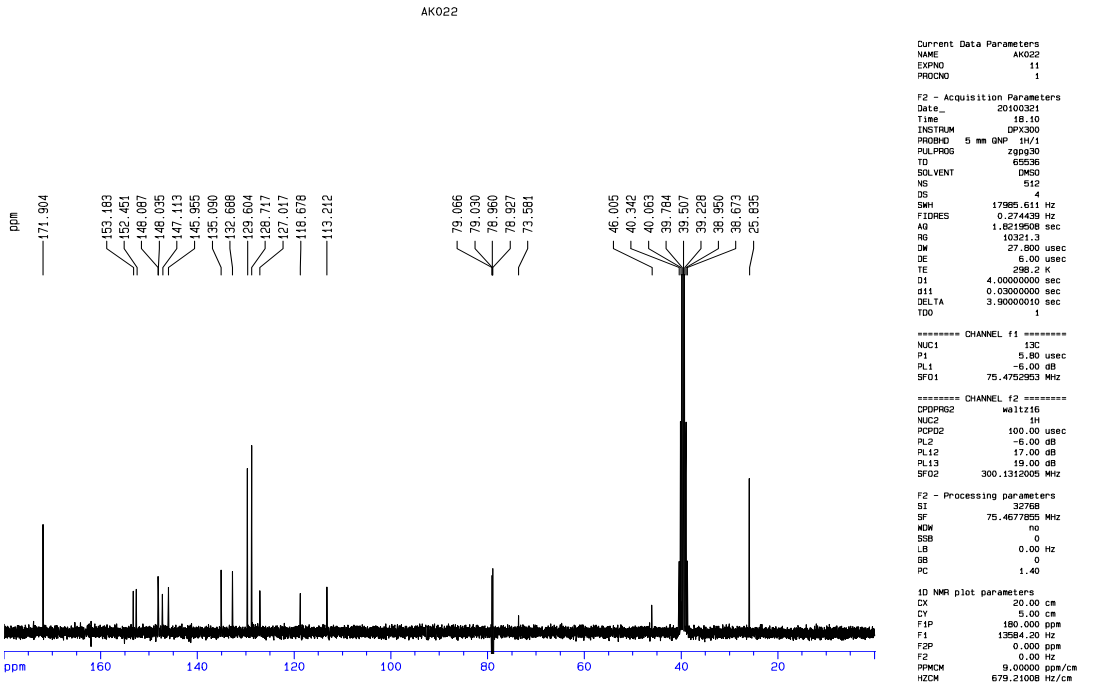
HR-MS Found 409.0948, calcd. for $C_{20}H_{16}ClN_5O_3$ 409.0942.

Anal.: Found C, 58.40; H, 4.09; N, 17.32. $C_{20}H_{16}ClN_5O_3$ requires C, 58.61; H, 3.94; N, 17.09.

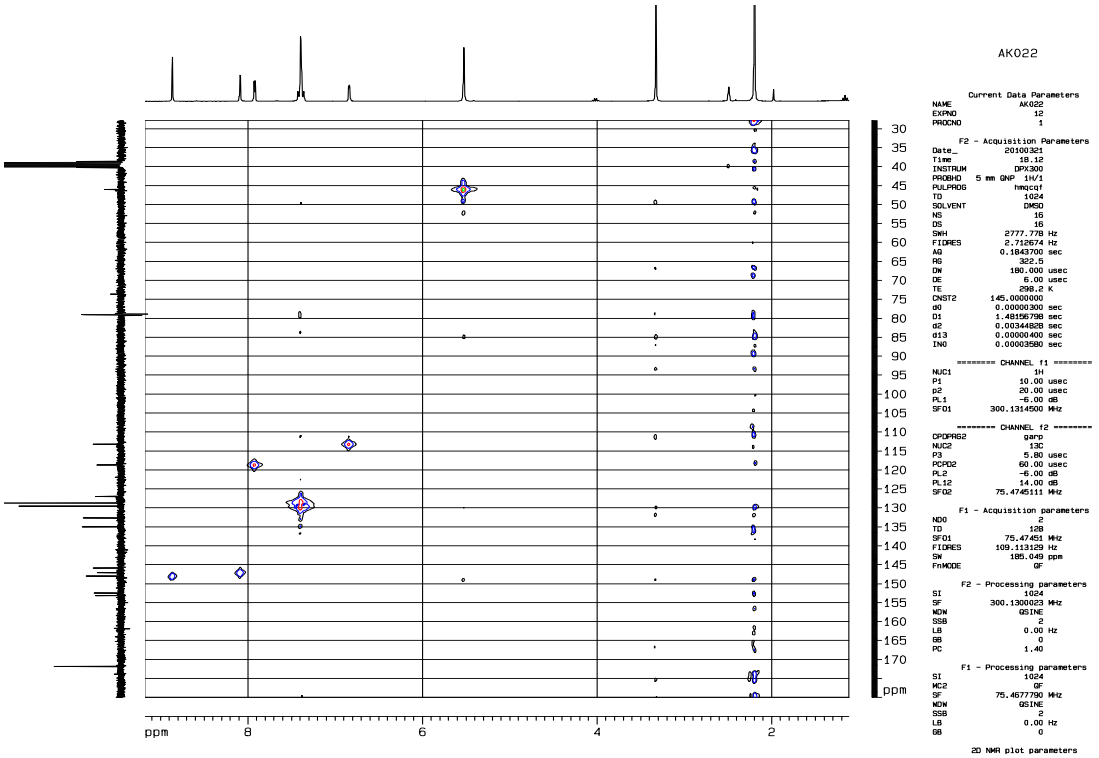
M.p. 199 ºC



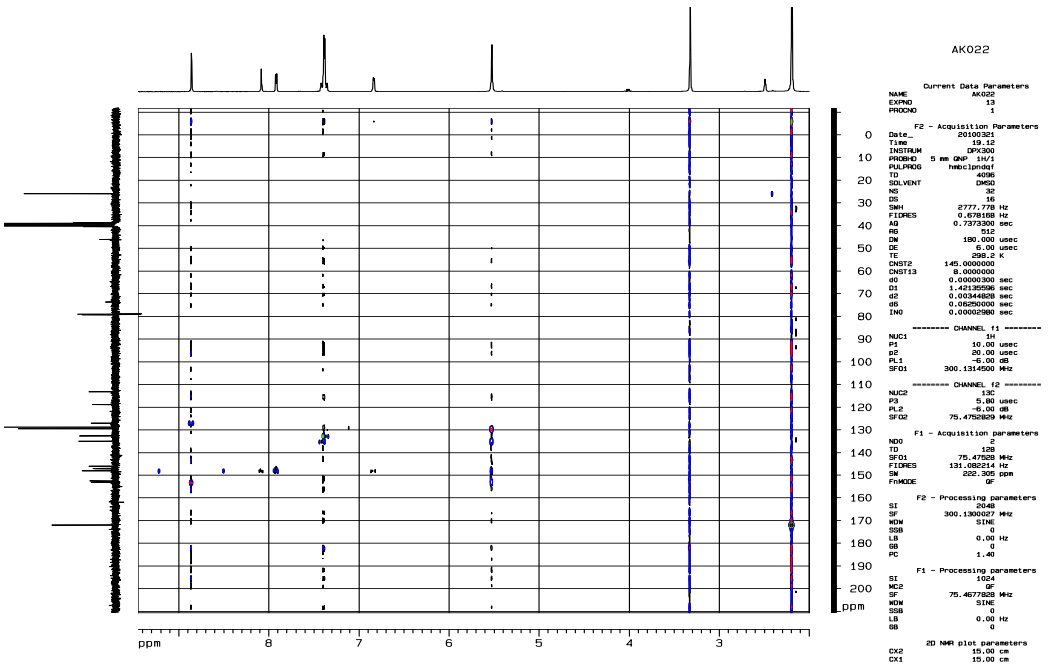
Spectrum 45. ^{1}H of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b).**



Spectrum 46. ^{13}C of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b).**

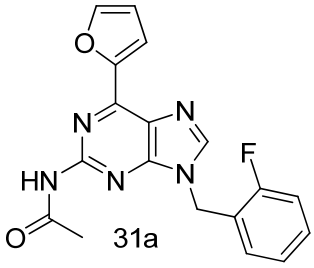


Spectrum 47. HMQC of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b).**

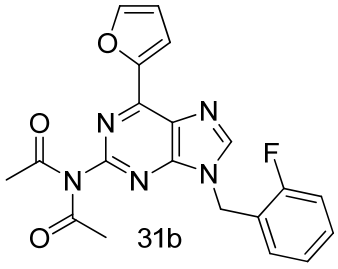


Spectrum 48. HMBC of *N*-acetyl-*N*-(9-(4-chlorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(30b)**

Synthesis of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide (31a) and *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide (31b)



31a



31b

Method 1

Acetic anhydride (0.22 mL, 2.30 mmol) was added to a stirred suspension of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** (81 mg, 0.26 mmol) and triethylamine (0.18 mL, 1.42 mmol) in toluene (2.60 mL) and the resulting mixture was heated at reflux for 24 h, cooled and evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (2:1) followed by ethyl acetate. This gave 50 mg (63 %) of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)** as a colourless powder.

Method 2

Acetic anhydride (0.46 mL, 4.90 mmol) was added to a stirred suspension of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** (160 mg, 0.50 mmol) and triethylamine (0.34 mL, 2.40 mmol) in toluene (5 mL) and the resulting mixture was heated at reflux for 24 h, cooled and evaporated *in vacuo.* The product was purified by flash chromatography on silica gel using ethyl acetate/hexane (1:1) followed by ethyl acetate and crystallized from ethyl acetate/hexane. This gave 4.5 mg (3 %) of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)** as a colourless powder.

Method 3

A stirred suspension of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** (190 mg, 0.60 mmol) in toluene (5 mL) was heated at reflux for 24 h. Acetic anhydride (0.57 mL, 6.0 mmol) was added. After additional 24 h reflux, the solution was evaporated *in vacuo.* The products were purified by flash chromatography on silica gel using ethyl acetate/hexane (1:2) followed by ethyl acetate/hexane (2:3). This gave 110 mg (59 %) of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)** as a colourless powder and 10 mg (4 %) of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b)** as colourless crystals.

Method 4

Acetic anhydride (0.57 mL, 6.0 mmol) was added to a stirred suspension of 9-(4-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-amine **(29a)** (190 mg, 0.60 mmol) in toluene (5 mL). The mixture was heated at reflux for 24 h, cooled and evaporated *in vacuo.* The products were purified by flash chromatography on silica gel using DCM/ethanol (98:2).

This gave 40 mg (21 %) of N-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9H-purin-2-yl)acetamide **(31a)** as a colourless powder and 80 mg (32 %) of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b)** as colourless crystals.

N-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a)**

^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.53 (br s, 1H, NH), 8.52 (s, 1H, H-8), 8.04 (dd, J = 1.7 Hz and 0.8 Hz, 1H, H-5 in furyl), 7.81 (dd, J = 3.5 Hz and 0.8 Hz, 1H, H-3 in furyl), 7.37-7.15 (m, 4H, CH in Ar), 6.80 (dd, J = 3.5 Hz and 1.7 Hz, 1H, H-4 in furyl), 5.48 (s, 2H, CH_2), 2.22 (s, 3H, CH_3).

^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.2 (CO), 159.9 (d, J_{CF} = 246 Hz, CF in Ar), 152.8 (C-5), 152.6 (C-2), 148.7 (C-2 in furyl), 146.3 (C-5 in furyl), 145.5 (C-8), 145.1 (C-6), 130.3 (d, J_{CF} = 3.4 Hz, CH in Ar), 130.1 (CH in Ar)[†], 124.7 (d, J_{CF} = 3.5 Hz, CH in Ar), 124.3 (C-4), 123.1 (d, J_{CF} = 14.5 Hz, C in Ar), 117.5 (C-3 in furyl), 115.4 (d, J_{CF} = 20.8 Hz, CH in Ar), 112.8 (C-4 in furyl), 40.4 (CH_2), 24.5 (CH_3).

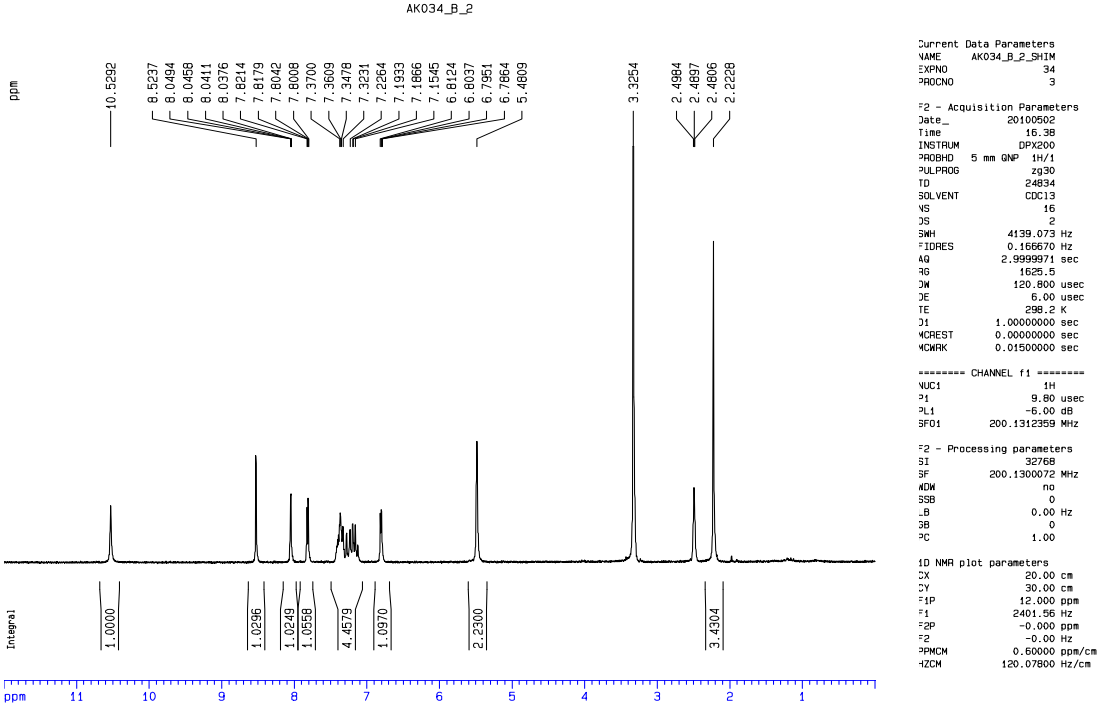
MS EI *m/z* (rel. %): 351 (85, M^+), 309 (100), 290 (6), 200 (28), 109 (97), 83 (8).

HR-MS Found 351.1135, calcd. for $C_{18}H_{14}FN_5O_2$ 351.1132.

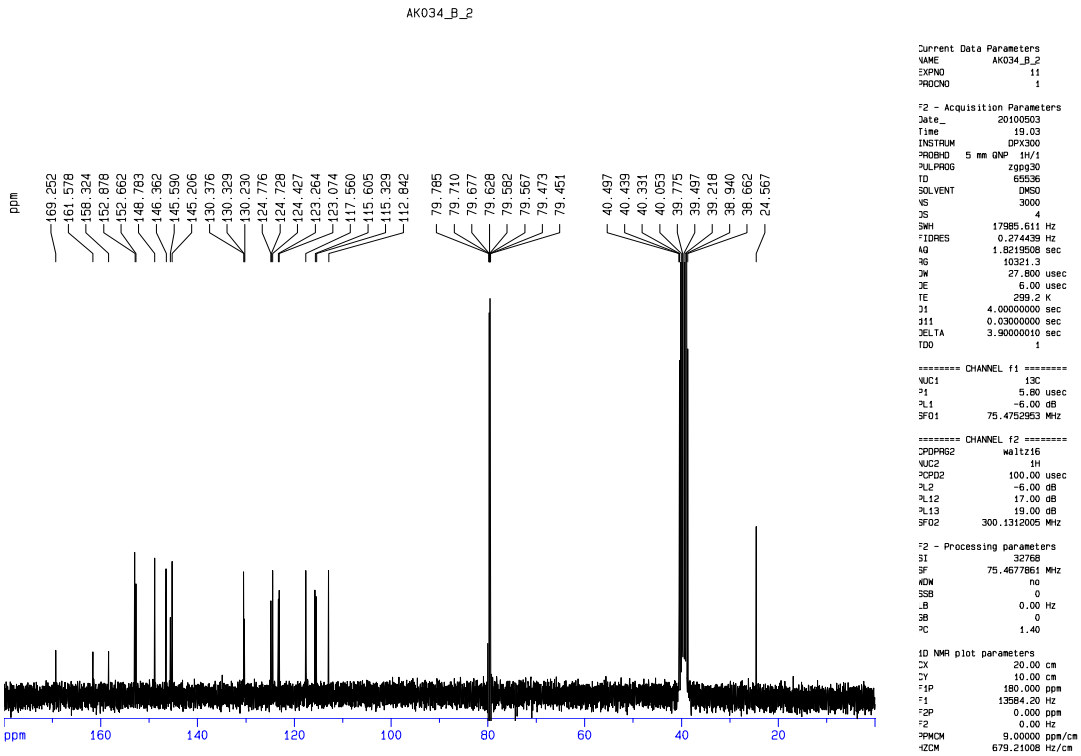
Anal.: Found C, 60.93; H, 3.45; N, 19.72. $C_{18}H_{14}FN_5O_2$ requires C, 61.53; H, 4.02; N, 19.93.

M.p. 194 ºC

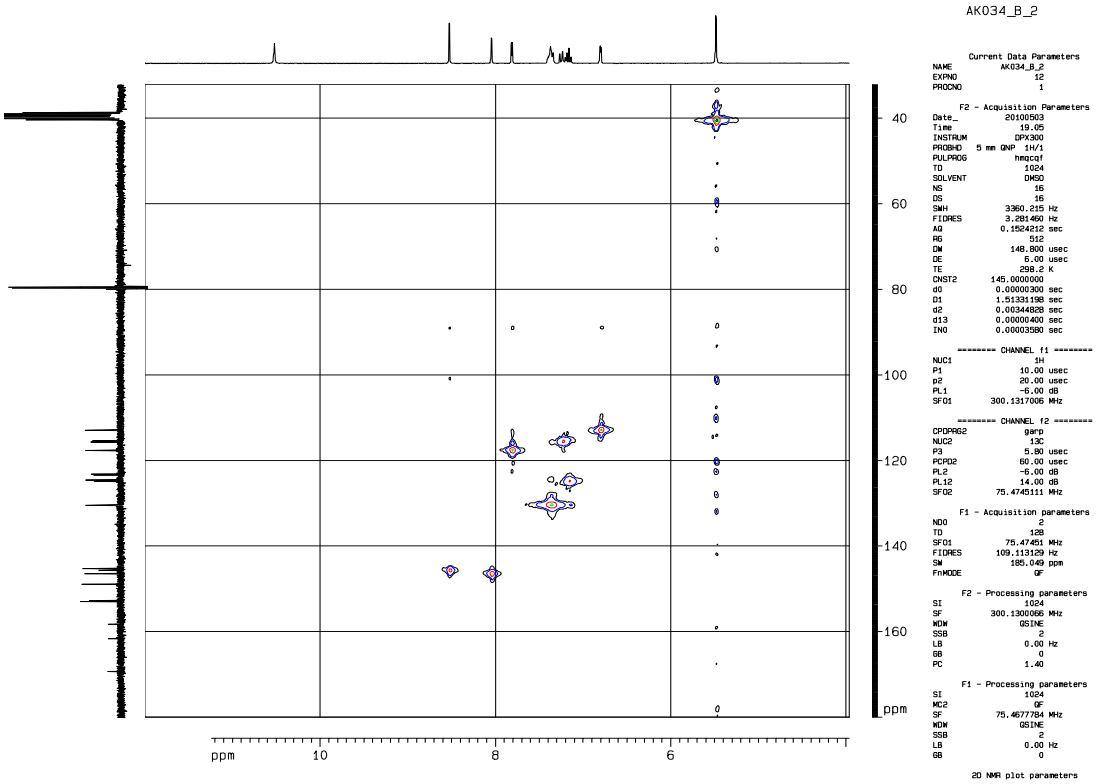
[†] Signals for the CH carbon in ^{13}C spectrum overlaps with another CH signal. It is not possible to calculate the coupling constant. Integration of the peak confirms that it is an overlap of signals.



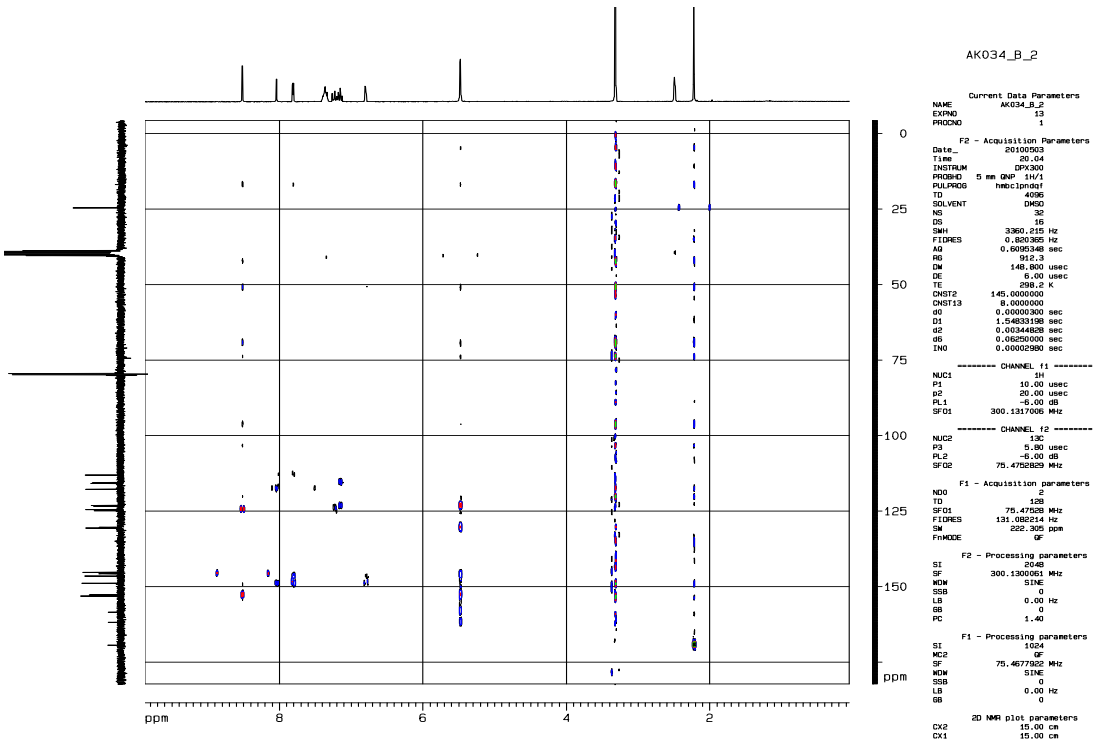
Spectrum 49. ^{1}H of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a).**



Spectrum 50. ^{13}C of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a).**



Spectrum 51. HMQC of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a).**



Spectrum 52. HMBC of *N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31a).**

N-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b)**

^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.80 (s, 1H, H-8), 8.08 (dd, J = 1.7 Hz and 0.8 Hz, 1H, H-5 in furyl), 7.92 (dd, J = 3.5 Hz and 0.8 Hz, 1H, H-3 in furyl), 7.36-7.16 (m, 4H, CH in Ar), 6.84 (dd, J = 3.5 Hz and 1.7 Hz, 1H, H-4 in furyl), 5.58 (s, 2H, CH_2), 2.17 (s, 6H, 2 × CH_3).

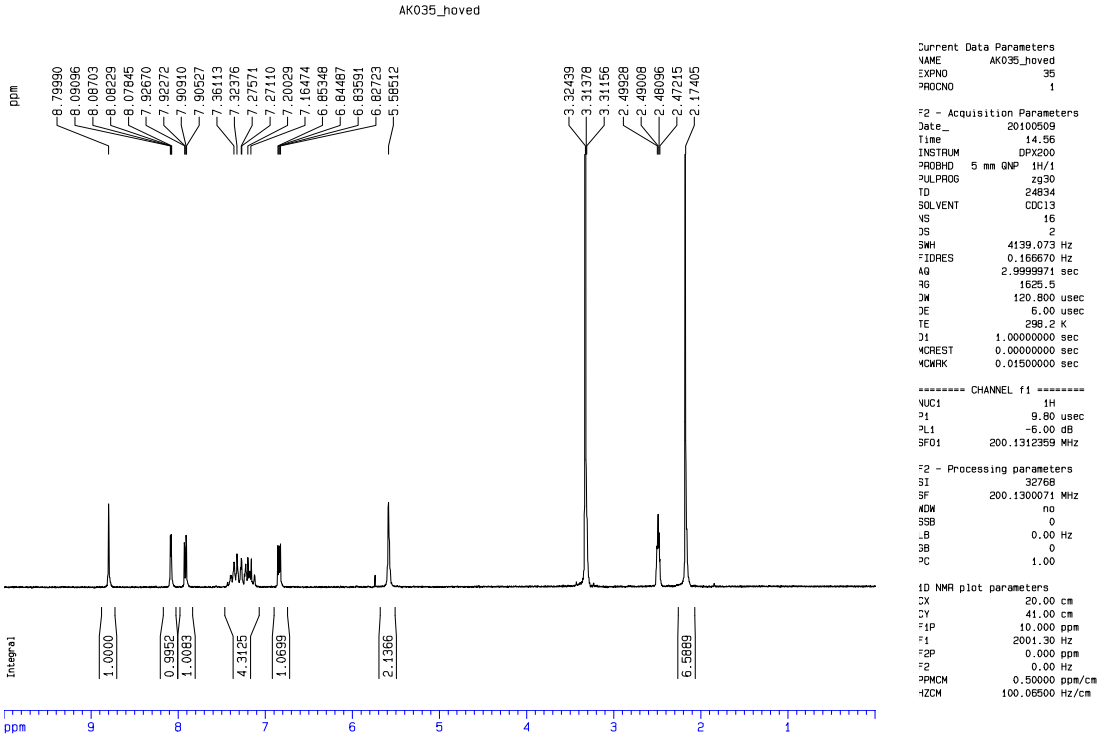
^{13}C NMR (DMSO-d_6, 75 MHz): δ 171.8 (2 × CO), 159.9 (d, J_{CF} = 246 Hz, CF in Ar), 153.1 (C-4), 152.4 (C-2), 148.0 (C-2 in furyl), 147.9 (C-8), 147.0 (C-5 in furyl), 145.9 (C-6), 130.4 (d, J_{CF} = 8.3 Hz, CH in Ar), 130.1 (d, J_{CF} = 3.5 Hz, CH in Ar), 126.8 (C-5), 124.7 (d, J_{CF} = 3.5 Hz, CH in Ar), 122.8 (d, J_{CF} = 14.5 Hz, C in Ar), 118.6 (C-3 in furyl), 115.5 (d, J_{CF} = 20.8 Hz, CH in Ar), 113.1 (C-4 in furyl), 41.0 (CH_2), 25.7 (2 × CH_3).

MS EI *m/z* (rel. %): 393 (12, M^+), 351 (59), 336 (86), 309 (65), 200 (16), 109 (100), 83 (9), 43 (18).

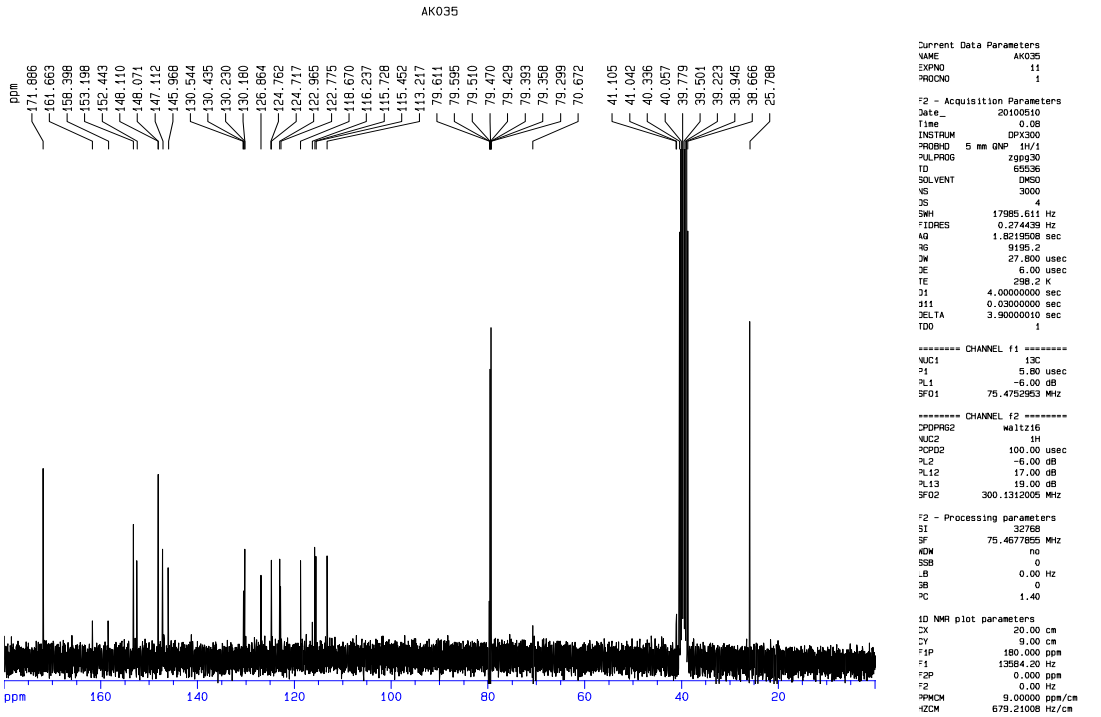
HR-MS Found 393.1240, calcd. for $C_{20}H_{16}FN_5O_3$ 393.1237.

Anal.: Found C, 60.78; H, 3.49; N, 17.61. $C_{20}H_{16}FN_5O_3$ requires C, 61.07; H, 4.10; N, 17.80.

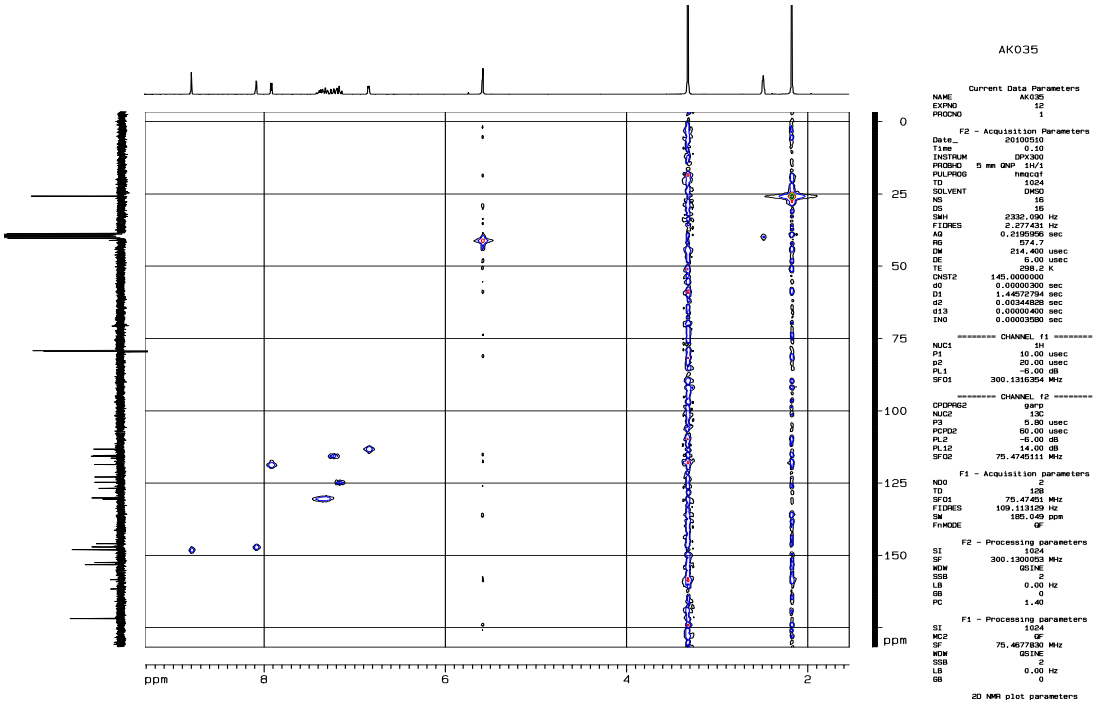
M.p. 178 ºC



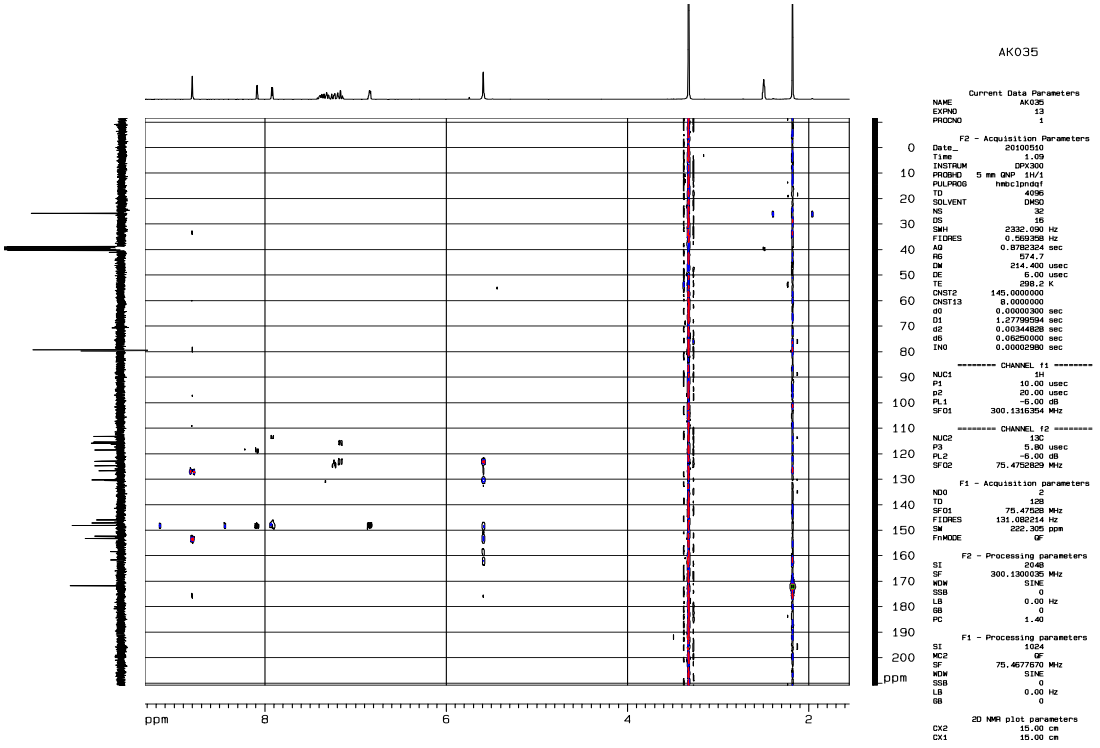
Spectrum 53. ^{1}H of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(11b).**



Spectrum 54. ^{13}C of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(11b).**



Spectrum 55. HMQC of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b).**



Spectrum 56. HMBC of *N*-acetyl-*N*-(9-(2-fluorobenzyl)-6-(furan-2-yl)-9*H*-purin-2-yl)acetamide **(31b).**

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